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Poster

658. Adult Neurogenesis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 658.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: 5T32DA007287-17

NIDA T32 Grant 3T32DA007287-18S1

John S. Dunn Foundation

University of Texas Medical Branch, Institute of Translational Sciences and Graduate School of Biomedical Sciences

Title: Ethanol consumption impacts adult neurogenesis in a sex, time, and region dependent manner as determined by genetic fate mapping

Authors: *E. L. MCGRATH, J. GAO, T. DUNN, B. S. KAPHALIA, K. T. DINELEY, K. A. CUNNINGHAM, P. WU;
UTMB, Galveston, TX

Abstract: Alcohol abuse is the third leading cause of preventable death in the United States, with more than 16.6 million adults over age 18 meeting the criteria for alcohol use disorders in 2013. It is well established that alcohol has detrimental effects on the brain and corresponds with deficits in cognitive and behavioral function. In many cases, damage incurred by long-term alcohol use is irreversible. Recently, alcohol has been shown to alter neural stem cells (NSCs) in the adult brain, however, little is known about how differences in brain region, sex, and duration of alcohol use can affect the response of NSCs. To better understand the full extent of these differences, we used an inducible transgenic mouse model to map the fate of adult endogenous NSCs following long-term alcohol treatment. Mice were ages 80-100 days at the start of the experiment and individually housed. They were fed a complete nutrient calorie-controlled liquid diet, which was measured daily, and were given *ad libitum* access to water. A subset of mice was harvested within the first two weeks and were used to evaluate the short term effects of alcohol use on NSCs. Short term was defined as maximum percent alcohol consumption for approximately 2 weeks or less, and long term treatment was defined as maximum percent alcohol consumption for greater than 3 weeks. At the experimental endpoint, mice were harvested and brain tissue underwent immunohistochemical staining, followed by confocal microscopy image analysis. We observed a distinct pattern of NSC behavior in the subventricular

zone of the lateral ventricle (SVZ), the sub-granular zone of the dentate gyrus (SGZ) and the tanycyte layer of the third ventricle (TL). Female mice display a significant decrease in NSC survival and neurogenesis over a shorter period of time in the SVZ when compared to male mice. In the TL, male mice exhibited a robust proliferation of NSCs with a unique morphological phenotype when consuming ethanol over a short period of time. This same effect was not seen in females. There was an increase in NSC proliferation and astrogliogensis in the SGZ following short and long term alcohol consumption in both male and female mice, which persisted through short-term into long-term consumption. We also observed that females tended to develop more severe symptoms, whereas males persisted with mild symptoms.

Disclosures: **E.L. McGrath:** None. **J. Gao:** None. **T. Dunn:** None. **B.S. Kaphalia:** None. **K.T. Dineley:** None. **K.A. Cunningham:** None. **P. Wu:** None.

Poster

658. Adult Neurogenesis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 658.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: DFG Grant KL 2805/1-1 to F.K.

Title: ACE2 activity sustains serotonin levels that mediate the running-induced effect on adult neurogenesis

Authors: *F. C. KLEMPIN[1], M. BADER[1], R. SANTOS[2], N. ALENINA[1];
[1]Max-Delbruck-Centrum (MDC), Berlin, Germany; [2]UFMG, Belo Horizonte, Brazil

Abstract: Running is a strong external effector that induces precursor cell proliferation in the adult mouse hippocampus. We have recently established that serotonin is the signaling factor that transduces physical activity into adult neurogenesis. While direct pathways for serotonin activity in brain function is becoming understood, less focus has been given on many potential peripheral signals that may cause pro-mitotic running effect. The main system in the body that regulates cardiovascular homeostasis is the renin-angiotensin system (RAS). Here, we explore the effect of the acute running stimulus on adult neurogenesis in conditions of deregulated RAS. Specifically, we took advantage of mice with genetic deletion of the principle regulating enzyme of the RAS, angiotensin (Ang) - converting enzyme (ACE) 2, and the Ang-(1-7) receptor Mas, and measured proliferation and differentiation of dentate gyrus precursor cells. ACE2 controls the transition from Ang II signaling, which is vaso-constrictive and pro-inflammatory to that of Ang-(1-7),

which is vasodilatory and anti-inflammatory. Recent studies suggest an additional role for ACE2 as an amino acid transporter, facilitating the absorption of large dietary neutral amino acids in the gut. In ACE2-deficient mice impaired tryptophan (Trp) absorption has been described accompanied by decreased levels of Trp and serotonin in the blood and brain. Since L-Trp is the precursor of serotonin, ACE2 could indirectly modulate brain serotonin levels that are in turn known to affect adult neurogenesis. Indeed, we observed decreased brain serotonin levels and no increase in the number of BrdU-positive cells following physical exercise in ACE2-deficient mice. We demonstrate that experimentally increased levels of Trp and serotonin could not rescue the effect, and show how other components of the RAS such as the G-protein coupled receptor Mas maybe involved in central effects mediated by a local RAS. Our data identify ACE2 activity as a novel pathway that is serotonin-independent but required for exercise-dependent modulation of adult neurogenesis. These experiments will impact our understanding of the cardiovascular network and it's role in translating physical exercise with brain cell genesis.

Disclosures: **F.C. Klempin:** None. **M. Bader:** None. **R. Santos:** None. **N. Alenina:** None.

Poster

658. Adult Neurogenesis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 658.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Support: FAU research grant

Title: Expression and function of Sox Neuro in the development of the *Drosophila* adult nervous system

Authors: *S. SINGH, K. DAWSON-SCULLY, J. R. NAMBU;
Biol. Sci., Florida Atlantic Univ., Jupiter, FL

Abstract: The mammalian Sry gene, a sex determining Y chromosome was discovered in 1990 and encodes a transcription factor with single high mobility group DNA binding domain. More than 20 transcription factors in humans and 8 in *Drosophila* share a related HMG domain with at least 50% identity to that of Sry. These Sox (Sry box) proteins bind to the minor groove of DNA and induce 70° to 90° bends to regulate chromatin structure and transcription initiation. *Drosophila* possesses 4 highly related Group B Sox genes SoxNeuro, Dichaete, Sox21a and Sox21b. Previous study has shown that Dichaete has a strong role in development, affecting processes that include differentiation of specific neuronal and glial cells, segmentation, hindgut

development, differentiation of imaginal discs. Dichaete is expressed in several clusters of neurons in the brain, including intermingled olfactory LNs and central complex neurons and important for the elaboration of the adult olfactory system. SoxN function is important for the formation of neural progenitor cells in *Drosophila* and evidence suggests that loss of SoxN function results in defects in the neuroblast formation. Interestingly, both SoxN and Dichaete have region-specific functions in CNS development as they both regulate dorsal/ventral partitioning of the embryonic neuroectoderm into specific columns. Evidence also suggests that SoxN and Dichaete function in a redundant manner in neuroblast formation and other developmental processes including neuroectoderm formation, central nervous system development, and sensory trichome formation. However, little is known about the expression and function of SoxN in the adult brain. This study focuses on identifying the expression patterns and function of SoxN in the development of the adult nervous system. Our data shows SoxN is expressed both in neurons and glia of the adult central brain. SoxN expression overlaps with Dichaete in the adult central brain. We are currently determining the precise identity of SoxN brain expressing cells. The result of this study will lead to a better understanding of Sox gene functions in both conserved and specific aspects of development. Sox proteins have essential developmental functions in many species, including key roles in sex determination, segmentation, neural patterning, differentiation of neurons and glia, and formation of eyes, bone, cartilage, heart, and craniofacial structures. Furthermore, Sox gene mutations are associated with a wide array of human congenital disorders and cancer. Thus, these studies on *Drosophila* Sox genes may illuminate conserved developmental functions in mammals and are relevant for human health.

Disclosures: **S. Singh:** None. **K. Dawson-Scully:** None. **J.R. Nambu:** None.

Poster

658. Adult Neurogenesis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 658.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: Berlin Institute of Healt

Title: Shedding light on serotonin in depression, and its linked role to adult neurogenesis

Authors: ***M. PETERMANN**[1], N. ALENINA[2], G. KRONENBERG[3], F. KLEMPIN[2];
[1]Max Delbrueck Ctr., Max Delbrueck Ctr. For Mol. Sci. In Helm, Berlin, Germany; [2]Max

Delbrueck Ctr. for Mol. Med., Berlin, Germany; [3]Dept. of Psychiatry and Psychotherapy, Charité - Univ. Med. Berlin, Berlin, Germany

Abstract: The fast progression of modern society is accompanied by a dreadful tribute - the risk to develop depression. Serotonin is well known as "molecule of happiness" and widely used in clinics. Yet, the neuromodulator is discussed to be the main target in major depression and studies into new mechanisms of antidepressants revived. By now, manipulation of serotonin is the preferred medical treatment with its flagship application of selective serotonin re-uptake inhibitors (SSRIs). However, not all patients respond to SSRIs, and SSR-enhancers (SSREs) attract clinical attention since it also improves the patient's mood. Yet, SSREs show inconsistency with the theory in that they decrease the availability of extracellular serotonin, a 'hypothesis' killer. Along with deregulation of central serotonin, a decline in hippocampal neurogenesis has been observed leading to depressive-like behavior. In turn, clinical improvement by antidepressant therapy goes along with a slow, temporal increase in adult neurogenesis. Whether antidepressant drugs act solely via manipulating serotonin levels it's on debate. In addition, drugs may affect other transmitter or brain functions. Recent studies even argue for placebo-mindset-changing actions. Current research is seeking to better define the mechanisms in the adult brain, and to target depression in more patients. We have recently developed a mouse model deficient in the central serotonin synthesizing enzyme tryptophan hydroxylase (TPH) 2 (Tph2-/- mice), and discovered no changes in baseline proliferation rates. Together with a second mouse model deficient in serotonin transporter (SERT mice), the supposedly main target for SSRIs, we tested antidepressant action in absence or altered serotonin signaling. The exciting genetic loss-of-function models are useful tools to unravel the underlying mechanisms of drugs targeting the serotonin system. The SSRI citalopram or fluoxetine, and the SSRE tianeptine were given over 21 days following BrdU injection to determine the effect on adult neurogenesis. Control wild type (WT) and littermates received no injection or were treated with saline solution. Our data confirm that chronic SSRI treatment increased the number of precursor cells in WT mice. Surprisingly, saline injection alone already increased BrdU-positive cells significantly in Tph2-/- mice, while no effect was observed in SERT animals compared to untreated Tph2-/-, and wild type groups, respectively. We further demonstrate distinct results following SSRE treatment. Our study sets an interesting point in depression research, and supports the development of alternative pathways other than via serotonin.

Disclosures: **M. Petermann:** None. **N. Alenina:** None. **G. Kronenberg:** None. **F. Klempin:** None.

Poster

658. Adult Neurogenesis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 658.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01MH099114

Title: The role of kainate receptors in regulating maturation of adult-born granule neurons

Authors: *Y. ZHU, A. CONTRACTOR;
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Abstract: The adult hippocampus generates continuous cohorts of newborn granule cells that undergo sequential neuronal maturation and incorporation into the existing network in the dentate gyrus. Increasing evidence has demonstrated that adult-born neurons in the dentate gyrus can impact numerous functions that involve learning and memory and other cognitive processes; therefore the mechanisms that regulate the maturation and synaptic integration of these neurons have been a subject of great interest. It is known that maturation of newborn neurons is dependent on neuronal activity and neurotransmitter signaling including both GABA and glutamate. Glutamate exerts its actions through both ionotropic and metabotropic receptors, and while there is evidence for some glutamate receptors affecting maturation and integration of newborn neurons, the role, if any, that the kainate receptor subfamily play in these processes is still unknown. However, it has been demonstrated that kainate receptors are present in neural progenitors harvested from the adult hippocampus, and kainic acid application induces an increase in cellular proliferation. Moreover, in the subventricular zone, kainate receptor activation decreases the migration speed of neuroblasts. Therefore, it is possible that kainate receptors may play a role in regulating newborn cell maturation in the dentate gyrus. In this study we employed retrovirus labeling of dividing neural progenitors to demonstrate that newly generated granule cells exhibited kainate receptor-mediated currents starting at 2-3 weeks. Comparison of the intrinsic properties of neurons from wild type and constitutive GluK2-/- mice, in which kainate receptor-mediated currents are completely eliminated, demonstrated that newborn neurons lacking GluK2 developed their mature-like intrinsic membrane properties at a faster rate than neurons recorded in littermate controls. A similar phenotype of accelerated maturation was also found in neurons subjected to retrovirus-mediated, conditional single cell deletion of the GluK2 subunit using a Cre recombinase dependent strategy. These data indicate that GluK2 signaling mediates a cell autonomous effect on inhibiting the development of mature intrinsic properties of adult-born neurons. Ongoing work is assessing how elimination of kainate receptors in newborn neurons affects the development of neuronal morphology and their integration into the hippocampal circuitry. Taken together these studies are beginning to reveal a novel role of kainate receptors in regulating neuronal maturation of adult-born granule cells in the hippocampus.

Disclosures: **Y. Zhu:** None. **A. Contractor:** None.

Poster

658. Adult Neurogenesis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH/NIEHS Grant RO1-ES008146

Title: The effect of intranasal manganese exposure on adult neurogenesis in the subventricular zone (svz)

Authors: ***V. LAI**[1], S. O'NEAL[1], W. ZHENG[2];
[2]Hlth. and Human Sci., [1]Zheng Lab., West Lafayette, IN

Abstract: Exposure to manganese (Mn) causes neurodegenerative manganism, with clinical signs and symptoms similar but not identical to idiopathic Parkinson's disease (IPD). In the healthy adult brain, neural stem/progenitor cells are produced in the subventricular zone (SVZ) and migrate towards the olfactory bulb (OB) along the rostral migratory stream (RMS). Cell migration following solvent exposure shows that these cells are capable of migrating to other areas of the brain in addition to the known destination of the OB. However, whether exposure to Mn, which is known to be mainly via the inhalation and accumulate in SVZ, causes any aberrant migration remained unknown. This study was designed to investigate the effect of intranasal Mn exposure on brain adult neurogenesis. Rats received intranasal instillations of 0.2 mg Mn/kg (low dose), 0.8 mg Mn/kg (high dose) as MnCl2, or equivalent volumes of saline (control), once daily for 14 days. During the last 4 days, animals received 100 mg BrdU/kg in order to label newly generated cells. Brain slices were then stained for BrdU and DCX, a marker of neuroblasts. Confocal quantification of BrdU signal in the selected regions of interest in the SVZ at 4x magnification revealed no significant differences between control (676.8± 260.9) and Mn treated groups; however, a significant difference between low dose (437.4 ± 168.8) and high dose (914.1 ± 371.2) groups was observed (n= 3-8; $p < 0.05$). Similar outcomes were also observed for DCX staining (243.1 ± 89.7 in the low-dose group vs. 463.2 ± 128.0 in the high dose group; n= 3-8; $p < 0.05$). In addition, BrdU signals were observed in the corpus callosum of three animals following intranasal Mn exposure. These data indicate that intranasal Mn exposure appears to alter the processes in the adult neurogenesis in SVZ and affect neural cell migration. Altered adult neurogenesis may contribute, at least partially, to the etiology of Mn-induced neurodegenerative disorder.

Disclosures: **V. Lai:** None. **S. O'Neal:** None. **W. Zheng:** None.

Poster

658. Adult Neurogenesis

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: The Ichiro Kanehara Foundation for the Promotion of Medical Science & Medical Care

Title: Deletion of cohesin decreases adult neurogenesis in the subventricular zone

Authors: ***Y. FUJITA**, T. YAMASHITA;
Dept Mol Neurosci, Grad Sch. Med, Osaka Univ., Suita, Osaka, Japan

Abstract: Cohesin consists of four essential subunits, Smc1, Smc3, RAD21/Scc1 and Scc3. They hold sister chromatids together from the time of DNA replication to the onset of their segregation. This function is important for proper chromosomal segregation and DNA repair. These observations indicate that cohesin complex is essential for cell cycle progression and cellular proliferation. Loss of cohesin function results in 'cohesinopathies' such as Cornelia de Lange syndrome (CdLS). One of the major clinical features of CdLS is mental retardation, suggesting that cohesin has a crucial function in the central nervous system. Although cohesin function on chromosome segregation is studied intensively, its function in the central nervous system is poorly understood. To examine the function of cohesin in adult neurogenesis, we conducted loss of function analysis. We examined the consequences of deletion of cohesin function in the SVZ localized nestin-positive cells. We found that adult neurogenesis is inhibited when cohesin function is removed from the SVZ localized nestin-positive cells. Inhibition of cohesin function decreased the number of GFAP-positive cells and DCX-positive cells in subventricular zone. These results indicate that cohesin regulates adult neurogenesis in subventricular zone.

Disclosures: **Y. Fujita:** None. **T. Yamashita:** None.

Poster

658. Adult Neurogenesis

Location: Hall A

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Program#/Poster#: 658.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Support: T32NS061788

AGO34989

Title: The role of klotho in adult neurogenesis

Authors: *A. M. LASZCZYK, S. FOX, D. NETTLES, G. D. KING;
Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: The age modulating protein klotho (KL) critically regulates pathways involved in both normal aging and disease pathogenesis. In mice, over-expression (KLOE) extends lifespan up to 30% while loss of protein expression in the knockout (KLKO) reduces lifespan 85%. KLKO mice exhibit rapid, premature onset of phenotypes typically associated with advanced human age including cognitive impairment. Brains of KLKO mice develop normally but hippocampal dependent cognitive impairment develops between the 6th and 7th week of life. New investigations reveal that increased KL expression enhances cognition. Reciprocal effects on cognition when KL expression level is up- or down- regulated validate the importance of KL brain function and suggest that KL plays an important role in the maintenance and/or function of hippocampal neurons. It remains unclear how KL acts to elicit these effects, however KL modulates signaling pathways in the periphery that are essential for neurogenesis. Age-related down-regulation of neurogenic signaling proteins results in decreased neurogenesis which contributes to cognitive decline; and we have identified altered performance in dentate-specific cognitive tasks in the KLKO. Thus, we hypothesize that KL regulates neurogenesis through modulation of neurogenic signaling pathways. We sought to determine how neurogenesis is modified by KL expression level, using KLKO and KLOE mice to identify which neurogenic cell populations are altered and how signaling pathway(s) in the neurogenic niche are modulated. Neuronal progenitor proliferation was assessed by Ki-67 immunohistochemistry (IHC) followed by stereological quantification. Changes in proliferation were detected dependent on level of KL expression. IHC for radial glia marker brain lipid binding protein identified changes to the number of stem cells. We examined doublecortin (DCX), in POMC-GFP/KLKO mice, as markers of early maturation and commitment to neural fate. IHC revealed that modulation of KL expression level affects maturation of immature neurons and profoundly alters both the number and the dendritic arborization of immature neurons. Investigations are ongoing to understand how maturation is changed by tracking cells from birth with 5-bromo-2-deoxyuridine (BrdU) to determine fate. Preliminary IHC for both BrdU and stage-specific markers support a maturation

deficit in the KLKO, suggesting new cells are capable of committing to the neuronal fate, but maturation is stalled before neurons fully mature. Studies are ongoing to examine the activity of several molecules in neurogenic signaling pathways to determine which pathways are modulated by KL.

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Poster

658. Adult Neurogenesis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 658.09/A9

Topic: A.01. Neurogenesis and Gliogenesis

Support: VA Merit

Title: NGF regulates adult neurogenesis through a $p75^{NTR}$-ERK-miRNA-9 axis

Authors: ***J. SHI**, S. M. MASSA;
Dept. of Neurol., Dept. of Veterans Affairs Med. Ctr. and Univ. of California, San Francisco, CA

Abstract: Recent studies have suggested that loss or dysfunction of neuronal progenitor cells (NPCs) in the hippocampal dentate subgranular zone (SGZ) may contribute to post-traumatic memory deficits. We previously found that LM11A-31, a small-molecule non-peptide p75 neurotrophin receptor (NTR) modulator, which biases $p75^{NTR}$ signaling towards survival, mimicking NGF under some conditions, increased proliferation of polysialylated neural cell adhesion molecule (PSA-NCAM)-expressing cells, enhanced neurogenesis in the hippocampus, and improved spatial memory after traumatic brain injury in rats. Further, in hippocampal NPC cultures, NGF increased proliferation and differentiation, as well as survival and promoted $p75^{NTR}$-dependent PSA-NCAM expression in these cells (Shi *et al*. Stem Cells 2013; 31:2561-2574). To further investigate the mechanisms by which NGF promotes NPC proliferation and differentiation, we examined the roles of factors that have been implicated in the control of NPC growing, including extracellular-signal-regulated kinases (ERK1/2) and miRNA-9. With lentivirus-mediated over-expression of miRNA-9 and antisense miRNA-9 in NPC, we found that enforced miRNA-9 expression decreased the growth rate of NPC and PSA-NCAM-expressing cells *in vitro*. Antisense miRNA-9 increased the growth rate of NPC, beginning after several passages. Our studies have revealed relationships between miRNA-9 and NGF in the growth of NPC cells--NGF down-regulated miRNA-9 by up to 1000-fold and this was partially reversed by $p75^{NTR}$ antibody. Concordant with its effects on NPCs, NGF increased levels of phosphorylation

of ERK1/2 by up to ~80% compared to vehicle control, and the increase is blocked by a $p75^{NTR}$ N-terminal antibody. In contrast, brain-derived neurotrophic factor (BDNF) had no effect on ERK1/2 phosphorylation, but $p75^{NTR}$Ab together with BDNF increased phosphorylation as well as total expression of ERK1/2. Interestingly, *in vivo*, TBI increased miRNA-9 expression in SGZ PSA-NCAM-expressing cells and LM11A-31 partially reversed these effects, decreasing miRNA-9 expression. Together, these findings suggest that NGF may regulate the proliferation and differentiation of NPC cells through a p75NTR-ERK-miRNA-9 axis.

Disclosures: **J. Shi:** A. Employment/Salary (full or part-time):; Stephen M. Massa, Department of Neurology, San Francisco VA Medical Center and University of California,. **S.M. Massa:** A. Employment/Salary (full or part-time):; Stephen M. Massa, Department of Neurology, San Francisco VA Medical Center and University of California,.

Poster

658. Adult Neurogenesis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Canadian Institutes of Health Research (CIHR)

Foundation Fighting Blindness (FFB) / Krembil Foundation

Title: Exogenous factors induce rod- and cone-specific progenitors from adult retinal stem cells

Authors: ***B. G. BALLIOS**[1], S. KHALILI[1], K. GRISÉ[1], L. DONALDSON[2], G. BERNIER[3], V. A. WALLACE[1], D. VAN DER KOOY[1];
[1]Univ. Toronto, Toronto, ON, Canada; [2]McMaster Univ., Hamilton, ON, Canada; [3]Maisonneuve-Rosemont Hospital, Ctr. de recherché, Univ. de Montréal, Montréal, QC, Canada

Abstract: Adult retinal stem cell (RSCs) derived from the ciliary epithelium (CE) of mice can give rise to all retinal cell types. Taurine, retinoic acid and FGF2/heparin (T+RA+FH) added to differentiating clonal RSC colonies increases the number of rods to 90% of all progeny. RSC progeny produce 10% rods when differentiated in 1%FBS+FH (pan-retinal conditions). We hypothesized that exogenous factors act on RSC progeny in an instructive, rather than permissive, manner to bias photoreceptor differentiation through the enrichment of photoreceptor lineage-specific progenitors. RSCs were clonally isolated from the CE of 4-6 week old mice. We used limiting dilutions (<1 clone / well) of a fluorescent retroviral construct to label individual

progenitor clones *in vitro*. Clonal retroviral labeling revealed enrichment in the percentage of rod-only clones between 1%FBS (13%) to T/RA (over 70%), without affecting clone size or overall cell survival. This strongly argues against selective survival of rod progenitors or differential survival of post-mitotic rods within a clone. Single cell sorting by fluorescence-activated cell sorting (FACS) for side-scatter intensity allowed isolation of non-pigmented and pigmented cells in wells, which were then treated for 28 d. Survival, clone size, and phenotype were assessed by immunocytochemistry. In 1%FBS, clones derived from single non-pigmented progenitors were distributed between non-rod and mixed clones, with a minority of rod-only clones (100% Rhodopsin-positive; n=4 of 28 clones). Clones derived from pigmented cells in 1%FBS never gave rise to rod-only clones. In T+RA conditions, all clones from non-pigmented progenitors (n=34) were rod-only clones, while those from pigmented progenitors (n=47 of 48) were almost all no-rod clones. Of note, one rod-only clone (the largest) was derived from a single pigmented cell in T+RA, suggesting potential neural lineage plasticity in a very early, pigmented progenitor. Survival rates of non-pigmented cell derived clones were similar in T+RA and 1%FBS. Similar experiments using Wnt, BMP4 and TGFβ inhibition increases the number of RSC-derived cones to >60% of all progeny. We have used similar clonal analysis to isolate cone-specific proliferative progenitors (100% cone arrestin+), as well as cone-specific reporter mice to purify populations of RSC-derived cones by FACS. This study marks an important step in the characterization of photoreceptor-specific progenitors - no markers exist and literature is divided on their existence *in vivo*. Our study suggests a critical role for exogenous signals instructing early lineage decisions between fate-restricted retinal progenitors.

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Poster

658. Adult Neurogenesis

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Topic: A.01. Neurogenesis and Gliogenesis

Support: CONACYT CB-2013-01 222 193

Title: Effect of Aβ oligomers in adult mouse hippocampal neural precursor cells (NPCs)

Authors: M. SILVA-LUCERO[1], *M. CARDENAS-AGUAYO[1], G. RAMIREZ-RODRIGUEZ[2], M. MERAZ-RIOS[1];

[1]CINVESTAV-IPN, Mexico, Mexico; [2]Div. of Clin. Investigations, Natl. Inst. of Psychiatry "Ramón de la Fuente Muñiz", Mexico, Mexico

Abstract: Amyloid beta (Aβ) peptide is a product of Amyloid Precursor Protein (APP) processing, that is in low abundance and it is produced lifelong in the healthy brain. It is produced by sequential action of a β and a γ-secretase on APP, generating a sequence of 39 to 43 amino acids. Aβ40 and Aβ42 are composed for 40 and 42 amino acids, respectively; and they represent the majority peptides of Aβ found in plasma, cerebrospinal fluid and senile plaques. The functional properties of Aβ peptides to date have not been completely elucidated, although a number of studies suggest that the peptides have a number of neurotrophic and neurotoxic properties. Physiologically low concentrations of Aβ could play a key role in regulating synaptic plasticity and improving cognitive functions, however the accumulation of high concentrations of Aβ, combined with the effects of age, could cause dysregulation and loss of synaptic function, which is one of the characteristics of Alzheimer's disease. Neurogenesis is an active and dynamic process that involves the proliferation, migration and maturation of new neurons. This process is tightly regulated by several factors such as the niche, neurotransmitters, growth factors and hormones. Several studies suggest that Aβ peptides are involved in the process of neurogenesis, however the results are controversial. All existing data indicate a direct relationship between the physicochemical characteristics of the peptides and their effects. Here we evaluated the effect of Aβ oligomers on hippocampal neurogenesis. To this end we perform the characterization of Aβ oligomers 42 and 40 through atomic force microscopy and Western blotting (4G8 Antibody). NPCs isolated from the hippocampal dentate gyrus of adult rodents, were treated for 24, 48 and 72 hours with different concentrations of oligomeric forms obtained by Klein's protocol (2002) (Aβ 1-40 and 1-42), then evaluate the effect on viability (LDH Kit), proliferation (BrdU labeling) and the expression of differentiation markers: glial markers (GFAP) and neuronal markers (βIII-Tubulin). We found significant increase on cell viability in NPCs treated with oligomers Aβ40 (at 0.5, 1 and 5 μM) at 72 hours, however there was no effect on proliferation in NPCs treated neither with Aβ40 nor Aβ42 oligomers. Regarding differentiation markers, we found an increased expression of GFAP and βIII-Tubulin markers for cells that were treated with Aβ40. We conclude that Aβ could have an effect on NPCs survival, because it increases cell viability but not proliferation. Furthermore Aβ40 might be favoring neuronal differentiation.

Disclosures: **M. Silva-Lucero:** None. **M. Cardenas-Aguayo:** None. **G. Ramirez-Rodriguez:** None. **M. Meraz-Rios:** None.

Poster

659. Cell Fate Mechanisms

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Topic: A.01. Neurogenesis and Gliogenesis

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Title: Global transcriptomic analysis of compensatory response to genetic perturbation of the notch signaling pathway

Authors: *A. HALLERAN, C. RATNAYAKE, C. GOLINO, B. RABE, M. MCDONOUGH, M. SAHA;
Col. of William and Mary, Williamsburg, VA

Abstract: As an organism progresses through development it encounters chemical, physical, environmental, and genetic stressors that it must overcome in order to develop appropriately. While the molecular mechanisms that govern normal embryonic development are relatively well understood, comparatively little is known about how embryos respond to perturbations over the course of development. However, knowledge of this process is critical for unraveling the embryonic origins of adult disease and for understanding the unique plasticity of embryonic tissues. Conserved across all metazoans, the Notch pathway is a juxtacrine signaling pathway that establishes a balance between differentiated and progenitor neural cells during neurogenesis. Overexpression of Notch signaling at the two-cell stage of Xenopus laevis embryos results in a severely reduced NBT expression phenotype by the early tailbud stage. However, as development progresses to the swimming tadpole stage, NBT expression approaches control levels. Through global transcriptome analysis of Xenopus laevis embryos in which Notch signaling was mis-expressed at the two-cell stage, we have identified potential regulators of this observed embryonic compensation.

Disclosures: **A. Halleran:** None. **C. Ratnayake:** None. **C. Golino:** None. **B. Rabe:** None. **M. McDonough:** None. **M. Saha:** None.

Poster

659. Cell Fate Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.02/A13

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant HD037932

Title: Regulation of Ptf1a to generate a balanced neural network in the spinal cord

Authors: ***B. MONA**, J. M. AVILA, D. M. MEREDITH, J. E. JOHNSON;
Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Generating the correct balance of inhibitory and excitatory neurons in a neural network is essential for normal functioning of a nervous system. The neural network in the dorsal spinal cord functions in somatosensation where it modulates and relays sensory information from the periphery. PTF1A is a transcription factor expressed in a subset of neural progenitor cells that specifies an inhibitory neuronal fate while suppressing the excitatory neuronal fate in the dorsal spinal cord as well as in the cerebellum and retina. Thus, the regulation of Ptf1a expression is critical for determining mechanisms controlling neuronal diversity in these regions of the nervous system. We have identified multiple regulatory sequences in the Ptf1a gene locus that have distinct functions in directing Ptf1a expression. A highly conserved 2.3 kb auto-regulatory enhancer is present 13.4 kb upstream of the Ptf1a coding region that activates transcription in all Ptf1a expressing domains. A 1.2 kb enhancer located 11.2 kb 3' of the Ptf1a coding region is sufficient to direct expression to the chick and mouse dorsal neural tube. Activity of this 1.2 kb enhancer depends on the integrity of a sequence motif that matches the consensus binding site for a Paired - homeodomain family transcription factor. Furthermore, through bioinformatic analysis and subsequent experimentation, SOX3 was also found to regulate the 1.2 kb enhancer activity. Thus, multiple enhancer elements responding to a combination of transcription factors direct Ptf1a-domain expression. By mutating these motifs in mouse, individually and in combination using the CRISPR-Cas9 system, the *in vivo* contribution of these regulatory elements for Ptf1a expression and function in regulating the balance of inhibitory and excitatory neurons in the nervous system is being tested.

Disclosures: **B. Mona:** None. **J.M. Avila:** None. **D.M. Meredith:** None. **J.E. Johnson:** None.

Poster

659. Cell Fate Mechanisms

Location: Hall A

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Program#/Poster#: 659.03/A14

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 NS075188

NIH F32 NS087719

Title: Dual function of suppressor-of-fused in cortical progenitors during mammalian corticogenesis

Authors: *O. R. YABUT[1], K. YOON[2], G. FERNANDEZ[1], T. HUYNH[1], S. J. PLEASURE[1]; [1]Neurol., Univ. of California, San Francisco, San Francisco, CA; [2]Col. of Biotech. and Bioengineering, Sungkyunkwan Univ., Suwon, Gyeonggi-do, Korea, Republic of

Abstract: Cortical progenitors in the embryonic neocortex are tightly regulated to generate the correct number and subtypes of projection neurons that populate the mature mammalian neocortex. In our study, we found that the intracellular protein, Suppressor of Fused (Sufu), plays dual roles during corticogenesis to regulate the specification and proliferation of cortical progenitors at different timepoints. Conditional deletion of Sufu in cortical progenitors at early stages of corticogenesis (E10.5) result in the ectopic activation of Sonic Hedgehog (Shh) signaling leading to the abnormal specification of Pax6+ and Tbr2+ progenitors and the progressive loss of Tbr2+ progenitors as corticogenesis progressed. These defects result in the loss of upper layer projection neurons and the abnormal specification of the remaining projection neurons in the postnatal neocortex. In contrast, conditional deletion of Sufu at mid-corticogenesis (E13.5) does not disrupt the specification of cortical progenitors and the projection neurons they generate. Rather, our preliminary studies indicate that loss of Sufu primarily cause an increase in cortical progenitor proliferation, particularly the Tbr2+ population, and appears to be independent of Shh signaling activity. Collectively, our findings indicate that Sufu regulates cortical progenitor fate specification at early stages and proliferation at later stages of corticogenesis. The diverse role of Sufu is likely due to significant molecular changes in cortical progenitors as corticogenesis progress. Thus, our findings prompt the need to further dissect the spatial and temporal genetic changes in cortical progenitors to determine how specific molecular networks control fate specification and proliferation during the course of cortical development.

Disclosures: O.R. Yabut: None. **K. Yoon:** None. **G. Fernandez:** None. **T. Huynh:** None. **S.J. Pleasure:** None.

Poster

659. Cell Fate Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.04/A15

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01 NS044080

Title: Assessing the septal contribution to olfactory bulb interneuron diversity and the role of Gsx2 in septal progenitors

Authors: *S. QIN[1], H. CHAPMAN[3], S. M. WARE[4], R. R. WACLAW[2,1], K. CAMPBELL[1]; [1]Developmental Biol., [2]Exptl. Hematology and Cancer Biol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Dept. of Cell Biol. and Human Anat., Univ. of California, Davis, Davis, CA; [4]Pediatrics and Med. and Mol. Genet., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Earlier studies have shown lateral ganglionic eminence (LGE) progenitors generate a number of distinct neural cell types, including olfactory bulb (OB) interneurons. OB interneurons are thought to originate from the dorsal LGE (dLGE) and septum starting at embryonic time points. These cells migrate rostrally toward the OB, where they radially migrate to populate the different layers including the granule cell layer (GCL) and the glomerular layer (GL). Although some studies have attempted to investigate the contributions of dLGE versus septum to the OB interneuron subtypes, no genetic tool has been used to address this question. In this study, we utilize Zic3-LacZ BAC transgene, which is highly enriched in the septal ventricular zone (VZ) progenitor cells and serves as a short-term fate map of their OB derivatives. We find that β-galactosidase-positive septal progenitor cells give rise to a subpopulation of OB interneurons that express Sp8 and Calretinin (CR) but not Calbindin or TH, largely within the GL. The homeobox gene Gsx2 is expressed in VZ progenitors of the developing ventral telencephalon, including the LGE and septum. While a number of studies have examined the role of this factor in LGE progenitors, its function in septal progenitors remains unclear. To address this, we conditionally inactivated Gsx2 in the septum, leaving it largely intact in the dLGE progenitors, by recombining the Gsx2-floxed allele with Olig2-cre. Our results indicate that conditional inactivation of Gsx2 in the septum, results in impaired generation of Sp8-positive neuroblasts as well as reduced cell proliferation within the septal subventricular zone (SVZ). Accordingly, we observed decreased numbers of septum-derived (i.e. β-galactosidase-positive) Sp8- and CR-expressing OB interneurons within the GL. In summary, our results support the notions that septum (already at embryonic stages) is an important source of Sp8- and CR-expressing OB interneurons in the GL. Moreover, we show that Gsx2 is required within septal progenitors for the normal formation of the septal SVZ and subsequent generation of septum-derived Sp8- and CR-expressing OB interneurons.

Disclosures: S. Qin: None. **H. Chapman:** None. **S.M. Ware:** None. **R.R. Waclaw:** None. **K. Campbell:** None.

Poster

659. Cell Fate Mechanisms

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.05/A16

Topic: A.01. Neurogenesis and Gliogenesis

Support: Fondecyt Project N° 1150200

Title: CoREST1 and CoREST2 express in primary culture of midbrain dopaminergic neurons

Authors: M. GONZÁLEZ, L. A. PEREIRA, *M. E. ANDRES;
Pontificia Univ. Catolica De Chile, Santiago, Chile

Abstract: CoREST is a family of transcriptional co-repressors formed by CoREST1 (CoREST, RCOR1), CoREST2 (RCOR2) and CoREST3 (RCOR3). The CoREST proteins form part of a transcriptional regulator complex, also comprising the histone demethylase LSD1/ KDM1A and the histone deacetylases 1 and 2 (HDAC1/2), here referred as the LCH complex. A variety of transcription factors interacts with members of CoREST family to bring the LCH complex to repress target genes during differentiation, regulatory and pathological processes. Recent work has given evidence that CoREST1 regulates the expression of genes of the dopamine neuronal phenotype during development. For instance, it was shown that CoREST1 binds to the promoters of tyrosine hydroxylase (TH) and dopamine transporter (DAT) genes. Through this interaction, CoREST1 maintains repressive epigenetic features and lower transcription of these genes, during dopamine neurons development. We have wondered whether CoREST2 and CoREST3 play a role in dopamine phenotype acquisition and maintenance in adulthood. Previously, we showed that the 3 CoRESTs express widely in adult rat brain. Here, we have extended the study of CoRESTs expression to cultured mesencephalic cells to learn whether all CoRESTs express in dopamine neurons during *in vitro* maturation. Cultured mesencephalic cells, obtained from rat embryonic day 14-18th were maintained during different length periods until 14 days *in vitro*. After fixing, the cells were incubated with specific antibodies against CoREST1, CoREST2 and TH. The results show that CoREST1 and CoREST2 express in all TH-positive cells during *in vitro* maturation. In addition, the data show that the expression of both CoRESTs decreases during maturation, suggesting that both CoREST1 and CoREST2 play a role in dopamine neuronal maturation. Funded by Fondecyt Project N° 1150200

Disclosures: M. González: None. **L.A. Pereira:** None. **M.E. Andres:** None.

Poster

659. Cell Fate Mechanisms

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: SOCCI Cancer Forum

Board of Gov. RMI Funding

Guerin Family

Smidt Family Foundation

Title: Ets factors regulate neural stem cell depletion and gliogenesis in perinatal development and glioma

Authors: H. PARK[1], R. LEVY[1], C. ANTONUK[1], J. MOLINA[1], M. DUTRA-CLARKE[1], A. AKHTAR[1], G. KIM[1], X. HU[2], S. BANNYKH[1], R. VERHAAK[2], M. DANIELPOUR[1], *J. J. BREUNIG[1];
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Abstract: As the list of putative driver mutations in glioma grows, we are just beginning to elucidate the combined effects of unhinged signaling pathways on the transformation of CNS cells. We have created a mosaic, autochthonous, glioma model that captures the first hours and days of gliomagenesis in more resolution than conventional genetically engineered mouse models of cancer (GEMMs). We provide evidence that disruption of the Nf1-Ras pathway in the VZ compartment at multiple signaling nodes uniformly results in rapid NSC depletion, progenitor hyperproliferation, and gliogenic lineage restriction. Abrogation of Ets subfamily activity, which is upregulated downstream of Ras, rescues these phenotypes and blocks glioma initiation. Further, we demonstrate the influence of Ets factors on early cell fate decisions of NSCs in normal perinatal brain. Thus, the Nf1-Ras-Ets axis might be one of the select molecular pathways that is hijacked for initiation and maintenance in glioma.

Disclosures: H. Park: None. **R. Levy:** None. **C. Antonuk:** None. **J. Molina:** None. **M. Dutra-Clarke:** None. **A. Akhtar:** None. **G. Kim:** None. **X. Hu:** None. **S. Bannykh:** None. **R. Verhaak:** None. **M. Danielpour:** None. **J.J. Breunig:** None.

Poster

659. Cell Fate Mechanisms

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Program#/Poster#: 659.07/A18

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant HD037932

Title: Understanding the role of Prdm13 in dorsal interneuron specification

Authors: *A. URUENA[1], J. CHANG[1], M. BORROMEO[1], R. KOLLIPARA[1], R. HAMMER[2], J. JOHNSON[1];
[1]Neurosci., [2]Biochem., UT Southwestern Med. Ctr., Dallas, TX

Abstract: After neural tube closure, there is an important stage of neurogenesis that is essential for producing the correct classes of neuronal populations. PTF1A is a transcription factor transiently expressed as neural progenitor cells become post-mitotic and begin to express neuronal specific gene programs. PTF1A specifies these cells to become GABAergic neurons (inhibitory neurons) while suppressing the glutamatergic neuronal program (excitatory neurons). A fundamental principle in bipotential cell fate decisions is the necessity to repress gene programs in the alternative fate. Recently, our lab identified PRDM13, a zinc finger containing transcription factor, as a direct downstream target of PTF1A that may serve this function in the inhibitory/excitatory neuron fate choice. Overexpression of PRDM13 in chick neural tube shows PRDM13 does indeed repress markers of the excitatory neuronal lineage. To explore PRDM13 function in more depth *in vivo*, and to expand these findings to regions outside the neural tube, a Prdm13GFP mutant mouse strain was generated that inserts a GFP coding region followed by a STOP codon. Prdm13GFP/GFP mice die neonatally, and at E10.5 show an increase in the dorsal neural tube excitatory neuron population at the expense of the inhibitory neurons. These phenotypes recapitulate that seen with Ptf1a null mice, and PRDM13 overexpression in chick neural tube. The Prdm13GFP mice have revealed additional insights into the function of PRDM13 and cell fate decisions in the developing spinal cord. First, PRDM13 negatively feedback regulates Ptf1a providing a mechanism for downregulating PTF1A as development progresses. Second, in contrast to the phenotype seen with Ptf1a mutants, late stage Prdm13 mutant embryos show only a partial loss of the inhibitory interneuron population, possibly due to the increased levels of PTF1A in these mutants. And finally, ChIP-Seq and RNA-Seq analysis of heterozygote versus homozygote Prdm13 mutants show that an important function of PRDM13 is to keep neuronal subtype specification genes for the ventral neural tube suppressed in the dorsal neural tube. Overall, this mouse model has placed PRDM13 in a pivotal role in the

specification of neuronal subtypes in the spinal cord, a function that will likely extend to the retina and cerebellum where PRDM13 is also present.

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Poster

659. Cell Fate Mechanisms

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.08/A19

Topic: A.01. Neurogenesis and Gliogenesis

Support: Wellcome DBT ECF to Bhavana Muralidharan - 500197-Z-11-Z

Intramural grant to Prof Shubha Tole from TIFR

Title: Mechanistic insights into regulation of the neuron-glia cell fate switch in the developing hippocampus by transcription factor Lhx2

Authors: ***B. MURALIDHARAN**[1], U. MAHESHWARI[2], S. PRADHAN[3], K. KARMODIYA[3], R. GUPTA[1], C.-H. BALAJI[1], V. KINARE[1], B. ROY[1], S. K. GODAVARTHI[1], D. CHAUHAN[1], U. KOLTHUR-SEETHARAM[1], S. GALANDE[3], S. TOLE[1];
[1]Dept. of Biol. Sci., Tata Inst. of Fundamental Res., Mumbai, India; [2]Friedrich Miescher Inst. for Biomed. Res., Basel, Switzerland; [3]Ctr. of Excellence in Epigenetics, Indian Inst. of Sci. Educ. and Res., Pune, India

Abstract: In the developing vertebrate nervous system, neural stem cells generate neurons first and then glia (astrocytes). The timing of this cell fate switch is regulated by interplay of intrinsic and extrinsic cues. We focused on the key issue of how progenitors are restricted from making glia during the neurogenic period. A previous study from our lab reported that LIM-homeodomain transcription factor Lhx2 is necessary and sufficient to suppress astrogliogenesis in the embryonic hippocampus (Subramanian et al., 2011). Loss of Lhx2 produces astrocytes from progenitors that would otherwise produce neurons. Overexpression of Lhx2 prolongs neurogenesis to generate neurons from progenitors that would otherwise give rise to astrocytes. The mechanism of this regulation remains unknown. We hypothesized that Lhx2 may regulate this switch by directly binding to and activating or repressing genes in neurogenic and/or gliogenic pathways. We analyzed genome-wide occupancy of Lhx2 by performing ChIP-seq using embryonic hippocampal tissue to identify potential Lhx2 target genes. We have identified

genes involved in progenitor proliferation, neurogenesis and gliogenesis. Ongoing work is focused on validating potential target genes using multiple approaches.

Disclosures: **B. Muralidharan:** None. **U. Maheshwari:** None. **S. Pradhan:** None. **K. Karmodiya:** None. **R. Gupta:** None. **C. Balaji:** None. **V. Kinare:** None. **B. Roy:** None. **S.K. Godavarthi:** None. **D. Chauhan:** None. **U. Kolthur-Seetharam:** None. **S. Galande:** None. **S. Tole:** None.

Poster

659. Cell Fate Mechanisms

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Wellcome DBT ECF to Geeta G IA/E/11/1/500402

TIFR intramural funds to Prof Shubha Tole

Title: Genetic interactions of Lhx2, Pax6 and Foxg1 in early the patterning of telencephalic domains

Authors: *G. GODBOLE[1], A. SHETTY[2], B. CHEN[3], G. MIYOSHI[4], G. FISHELL[4], S. TOLE[1]; [1]DBS, TIFR, Mumbai, India; [2]Harvard Univ. Dept. of Stem Cell and Regenerative Biol., Harvard Univ. Dept. of Stem Cell and Regenerative Biol., Cambridge, MA; [3]Molecular, Cell and Developmental Biol., Univ. of California Santacruz, California, CA; [4]Dept. of Physiol. and Neuroscience,, NYU Neurosci. Institute, NYU Langone Med. Ctr., New York City, NY

Abstract: The early telencephalic neuroepithelium consists of the cortical primordium flanked by two non-cortical structures, the hem medially, and the antihem laterally (Grove et al 1998, Assimacopoulos et al 2003). Three transcription factors act as early regulators of telencephalic patterning and control the formation of these structures: LIM-homeodomain family member Lhx2; winged helix factor Foxg1; paired domain and homeodomain containing factor Pax6. Both Foxg1 and Lhx2 supress hem fate (Muzio and Mallamaci 2005, Mangale et al 2008). Lhx2 also suppresses antihem fate (Mangale et al 2008), whereas Pax6 is necessary for antihem formation (Assimacopoulos et al 2003). When Lhx2 function is disrupted, the cortical neuroepithelium taken on hem and antihem identity, as a result of which these two structures expand in the *Lhx2* mutant (Mangale et al 2008). We asked whether Pax6 is required for the expanded antihem in the *Lhx2* mutant. Furthermore, we tested whether Lhx2 and Foxg1 interact to restrict the hem to its

normal location. Finally, using *CreER* and low dose tamoxifen, we examined the critical period for each of these factors in regulating hem and antihem fate. Our results reveal interesting interactions between these three early regulators of dorsal telencephalic patterning, underscoring their importance in the development of the cortical primordium.

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Poster

659. Cell Fate Mechanisms

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.10/A21

Topic: A.01. Neurogenesis and Gliogenesis

Support: EKFS 2013_A253

Title: Decoding subpallial neuronal diversity by single cell transcriptomic profiling

Authors: ***J. SYMMANK**[1], D. PENSOLD[1], N. HAAG[2], G. SALINAS-RIESTER[3], T. LINGNER[3], C. POMMERENKE[3], F. LUDEWIG[3], T. PIELER[3], G. ZIMMER[1];
[1]Inst. for Human Genet., [2]Inst. for Biochem. I, Univ. Hosp. Jena, Jena, Germany; [3]Dept. of Developmental Biochem., Univ. of Goettingen, Goettingen, Germany

Abstract: Most of the neuronal diversity in the mature telencephalon derives from progenitor cells of the subpallium giving rise to the astonishing variety of cells destined for the globus pallidus, septum, olfactory bulb, amygdala, striatum, hippocampus and the cerebral cortex. Despite a certain spatial and temporal bias regarding the generation of particular neuronal subtypes fated for the diverse destinations, most subpallial subdivisions like the medial and lateral ganglionic eminences (MGE and LGE), the caudoventral MGE (cvMGE), different regions of the preoptic area (POA) and septal anlagen represent mosaic structures contemporaneously generating various neuronal subpopulations. For the MGE and LGE molecular differences vastly correlate with the morphological appearance. In turn, subdivisions located ventrally of the MGE including pallidal septal domains, the cvMGE and the POA are partly continuous merging into each other. Moreover, the cvMGE, which is considered as a particular sub-domain, shares expression profiles with the MGE (like Lhx6 and Nkx2.1) as well as with septal subdivisions (Garcia-Lopez 2008). Similarly, the POA shares common features with septal and cvMGE-related tissue like Nkx2.1 expression (Flames et al., 2007). This complicates the exact demarcation of the different domains and the research on the fate of the

particular subdivisions. Additionally, the preoptic area consist of several subdomains like the POC, POB and lateral POA, which are suggested to distinctively give rise to certain neuronal subsets (Buspesh et al., 2011). The insufficient demarcation of the different domains complicates the research on the fate of the particular subdivisions. For this, we aimed to profile neuronal subsets generated in the POA and find candidate biomarker discriminating POA-derived cells from AEP and septal derivates by applying Nanostring nCounter based single cell transcriptome analysis.

Disclosures: **J. Symmank:** None. **D. Pensold:** None. **N. Haag:** None. **G. Salinas-Riester:** None. **T. Lingner:** None. **C. Pommerenke:** None. **F. Ludewig:** None. **T. Pieler:** None. **G. Zimmer:** None.

Poster

659. Cell Fate Mechanisms

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.11/A22

Topic: A.01. Neurogenesis and Gliogenesis

Support: Student Summer Scholars- Grand Valley State University

Title: Nato3 is sufficient to drive Lmx1b expression in the developing neural tube

Authors: **N. HUISINGH**[1], D. MARTINEZ[2], J. STRAIGHT[2], *M. K. TAYLOR[3]; [1]Biomed. Sci., [2]Cell and Mol. Biol., Grand Valley State Univ., Allendale, MI; [3]Biomed. Sci., Grand Valley State Univ., Grand Rapids, MI

Abstract: The developing chick embryo has multiple organizing centers which are important for the correct development of the neural tube. Nato3 is a bHLH transcription factor that is endogenously expressed in one of these, the floor plate region. This region also gives rise to dopaminergic neurons which are affected in Parkinson's disease through the coordinated expression of multiple transcription factors, including Lmx1b. Nato3 has a broad and not fully understood role in the proliferation and differentiation of stem cells in the neural tube. Here, we show that overexpression of Nato3 promotes Lmx1b expression in the neural tube. Nato3 was transfected using in ovo electroporation and monitored using a bicistronic EGFP reporter expression vector and the observed effects were characterized using immunohistochemistry. These data demonstrate that Nato3 can drive Lmx1b expression in the neural tube.

Disclosures: **N. Huisingh:** None. **D. Martinez:** None. **J. Straight:** None. **M.K. Taylor:** None.

Poster

659. Cell Fate Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.12/A23

Topic: A.01. Neurogenesis and Gliogenesis

Support: DFG ZI 1224/4-1

Title: A NanoString-based single cell approach for transcriptomic profiling of embryonic interneuron subtypes

Authors: *D. PENSOLD[1], J. SYMMANK[1], N. HAAG[2], G. SALINAS-RIESTER[3], T. LINGNER[3], C. POMMERENKE[3], F. LUDEWIG[3], T. PIELER[3], G. ZIMMER[1];
[1]Inst. for Human Genet., [2]Inst. for Biochem. I, Univ. Hosp. Jena, Jena, Germany; [3]Dept. of Developmental Biochem., Univ. of Goettingen, Goettingen, Germany

Abstract: The inhibitory actions of GABAergic cortical interneurons are crucial for proper cerebral information processing and impairments in interneuron development contribute to the pathophysiology of neuropsychiatric disorders like epilepsy, autism and schizophrenia. As the reversal of disrupted molecular deficits of neurodevelopmental disorders restores proper function even by treatments in adults, decoding determinants of interneurons maturation holds great promise for therapy strategies. The interneuron generating tissue represent a mosaic, contemporaneously generating various neuronal subtypes destined for several brain regions. This requires single cell resolution to decode the transcriptional control of cortical interneuron diversity and development. Single cell transcriptome analysis presupposes the availability of robust, quantitative methods, combining the suitability for analysis of even low copy number transcripts with the processing of larger sample sizes in a short time. Here, we applied the direct and sensitive NanoString nCounter technology to establish and validate an improved PCR-based strategy for quantitative and qualitative global single cell transcriptome analysis, enabling multiple analytical runs. We were able to profile distinct progenitor and postmitotic subpopulations from the interneuron generating medial ganglionic eminence (MGE). We further found new candidate for MGE-derived neuronal subsets. The identification of such lineage-specific/defining molecular markers is crucial for fate-mapping studies of distinct interneuron subpopulations.

Disclosures: **D. Pensold:** None. **J. Symmank:** None. **N. Haag:** None. **G. Salinas-Riester:** None. **T. Lingner:** None. **C. Pommerenke:** None. **F. Ludewig:** None. **T. Pieler:** None. **G. Zimmer:** None.

Poster

659. Cell Fate Mechanisms

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.13/A24

Topic: A.01. Neurogenesis and Gliogenesis

Support: Medical Research Council (MRC) Studentship

Title: NeuroD6 is required for the survival of midbrain dopaminergic neurons projecting to the intermediate lateral septum

Authors: *S. KHAN[1], A. TRUCKENBRODT[1], S. STOTT[1], A. CHABRAT[2], M. LÉVESQUE[2], M. UNGLESS[3], S.-L. ANG[1];
[1]The Francis Crick Inst., London, United Kingdom; [2]Ctr. de recherche de l'Institut universitaire en santé mentale de Québec, Québec, QC, Canada; [3]MRC Clin. Sci. Centre, Fac. of Medicine, Imperial Col. London, London, United Kingdom

Abstract: Midbrain dopaminergic (mDA) neurons represent a heterogeneous pool of neurons that differ in their projection pattern, connectivity to target areas, synaptic inputs and outputs that impact on their function. Here, we report the identification and characterisation of a novel subset of mDA neurons of the VTA that expresses the basic helix-loop-helix transcription factor, neurogenic differentiation factor-6 (NeuroD6). By combining a *Cre* knock-in strategy and a loss-of-function approach in mice, we have characterised this mDA neuronal subset at late embryonic, early-postnatal and adult stages showing that they form a subset of the Calbindin$^+$ and Raldh1a$^+$ mDA subpopulation of the VTA. NeuroD6$^+$ mDA neurons are localised specifically in the interfasicular nucleus (IFN), dorsal and ventral paranigral nucleus (PN) and the lateral parabrachial nucleus (PBP) of the medial VTA. In NeuroD6 mutants, 32% of the total NeuroD6-expressing mDA cells are lost within the medial VTA due to cell death as shown by TUNEL analysis. Furthermore, retrograde tracing experiments using fluorogold injected into the septum have demonstrated that NeuroD6-expressing mDA neurons specifically project to two distinct septal regions, the dorsal lateral septum (LSD) and intermediate lateral septum (LSI). In the absence of NeuroD6, specific loss of mDA axonal projections occurs within the LSI, while, axon projections of mDA neurons to the LSD remain unaffected in NeuroD6 mutants. Altogether, our results demonstrate that NeuroD6 labels a unique population of mDA neurons projecting to the LSD and LSI, but is only required for the survival of the latter neurons.

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Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Student Summer Scholars

Title: Comparison of the effects of mouse, chick and human nato3, a bhlh transcription factor, on floor plate marker expression in the developing neural tube

Authors: *D. DOYLE, N. HUISINGH, S. DURHAM, M. TAYLOR;
Grand Valley State Univ., Allendale, MI

Abstract: The Nato3 transcription factor from *Gallus gallus* has a unique sequence of 20 amino acids in its N-terminal domain when compared to highly conserved Nato3 sequences from seven other species. Due to this difference there may be unique effects from the *Gallus gallus* Nato3 as compared to the other species. In order to characterize differences between Nato3 from different species in the developing neural tube, we overexpressed Nato3 from *Mus musculus*, and did not see an upregulation of HNF3β, as reported with Nato3 from *Gallus gallus*. Currently we are testing the overexpression of Nato3 from the *Gallus gallus* and *Homo sapiens* to directly compare the effects of Nato3 from multiple different species.

Disclosures: D. Doyle: None. **N. Huisingh:** None. **S. Durham:** None. **M. Taylor:** None.

Poster

659. Cell Fate Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.15/A26

Topic: A.01. Neurogenesis and Gliogenesis

Support: NWO vici Grant 86.09.002

Title: Epigenetic mechanisms in midbrain development

Authors: *H. V. HEESBEEN, M. P. SMIDT;
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Abstract: Keywords: midbrain development, histone modification, epigenetics Brain development depends on specifically regulated gene-expression and cases of neurodevelopmental disorders have been related to epigenetic aberrations. Genome wide expression profiling in time has shown that specific components of histone methylation complexes are temporary up-regulated during neuronal terminal differentiation and not equally distributed in the brain. In order to understand this epigenetic control in midbrain development we have developed a set of tools to pinpoint the consequences of altered histone methylation. These tools allow ablation of one specific histone mark in time during midbrain development *in vivo*. Initial analysis of the corresponding histone mark indicates that maintenance of this mark is essential through active methylation events even without a known demethylase. Moreover, our initial results indicate a role for histone methylation in regulating specific neurodevelopmental features, including neuronal outgrowth.

Disclosures: H.V. Heesbeen: None. **M.P. Smidt:** None.

Poster

659. Cell Fate Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 659.16/A27

Topic: A.01. Neurogenesis and Gliogenesis

Title: A novel role played by Mecp2 during early neocortical progenitors proliferation and differentiation

Authors: *C. COBOLLI GIGLI[1], F. BEDOGNI[1], L. SCARAMUZZA[1], R. ROSSI[2], C. KILSTRUP-NIELSEN[3], N. LANDSBERGER[1];
[1]Neurosci., San Raffaele Hosp., Milano, Italy; [2]Inst. Nazionale Genetica Molecolare "Romeo ed Enrica Invernizzi", Milan, Italy; [3]Univ. of Insubria, Varese, Italy

Abstract: Mutations in the X-linked Mecp2 gene cause the autism spectrum disorder Rett syndrome (RTT). RTT affected children experience a first phase of apparently normal development lasting 6-18 months, followed by a rapid regression after which RTT typical traits (autistic features, seizures, ataxia and stereotypical hand movements) become evident. Given the timing of RTT onset, most of the studies in the field investigated the role of Mecp2 in maturity, roughly considering the embryonic development phase normal. However, different evidences of

the presence of subtle symptoms already at birth have been recently produced, suggesting a possible role for Mecp2 even during the earliest phases of development. Starting from these observations, we investigated the consequences of lack of Mecp2 on the embryonic development of the cerebral cortex at E15, when cortical progenitors are still proliferating giving rise to other progenitors and neurons. First of all we highlighted that Mecp2 in the developing cortex is expressed not only by post-mitotic neurons, as previously demonstrated, but also by cycling progenitors. Moreover, a role for Mecp2 in cycling cells is strongly suggested by the fact that its absence alters different pathways related to cell cycle, as demonstrated by our transcriptional analysis on embryonic cortexes. Interestingly, we show that the proliferation of Mecp2 null progenitors cultivated *in vitro* is impaired as well as their post mitotic output. Given these evidences we analyzed the *in vivo* dynamics balancing apical (Pax6 positive) and basal progenitors (Tbr2 positive): our experiments show that the identity of the two populations is not properly defined in the Mecp2 null samples. Our data demonstrate that lack of Mecp2 affects the development of the cerebral cortex from early stages never so far analyzed in deep details. We therefore suggest that the etiopathogenesis of RTT includes early stages of development during which Mecp2 plays a role that precedes its documented involvement in maintenance of mature neuronal systems later in life.

Disclosures: **C. Cobolli Gigli:** None. **F. Bedogni:** None. **L. Scaramuzza:** None. **R. Rossi:** None. **C. Kilstrup-Nielsen:** None. **N. Landsberger:** None.

Poster

659. Cell Fate Mechanisms

Location: Hall A

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Program#/Poster#: 659.17/A28

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 NS044080

Title: The role of Gsx2:Ascl1 protein-protein interactions on progenitor maturation in lateral ganglionic eminence (LGE) progenitors of the mouse

Authors: *K. ROYCHOUDHURY, M. NAKAFUKU, B. GEBELEIN, K. CAMPBELL; Developmental Biol., Cincinnati Children's Hosp., Cincinnati, OH

Abstract: Homeobox and basic helix loop helix (bHLH) transcription factors play critical roles in Progenitor maintenance vs differentiation in the ventricular and subventricular zones of the embryonic telencephalon. Within progenitors of the lateral ganglionic eminence (LGE), Gsx2

helps maintain progenitors in an undifferentiated state while Ascl1 plays an important role in progenitor maturation and neurogenesis. In an effort to characterize the molecular mechanisms underlying progenitor maturation in the LGE, we studied the protein-protein interactions of Gsx2. We found that Gsx2 and Ascl1 directly interact with each other at the protein level in a yeast 2-hybrid assay and in the embryonic mouse telencephalon. Using proximity ligation techniques, we have identified a population of ventricular zone progenitors in the LGE where Ascl1 and Gsx2 directly interact, *in situ*. We mapped the interaction domains on each of these proteins and found that a region C-terminal of Gsx2's homeodomain interacts with amino acid residues in the second helix of the bHLH domain of Ascl1. This interaction with Gsx2 inhibits Ascl1's ability to bind the E-box sequence in EMSAs. Moreover, this interaction inhibits heterodimerization of Ascl1 with E-proteins that are critical for its transcriptional activity. We are currently studying the effects of deleting Gsx2 in Ascl1-expressing LGE cells (using Ascl1-creER) on progenitor maturation. Moreover, we plan to use single cell RNAseq techniques to analyze the transcriptome of LGE progenitors that either co-express Gsx2 and Ascl1 or only express one of these genes, to identify distinct downstream effectors of these factors within the LGE lineage.

Disclosures: K. Roychoudhury: None. **M. Nakafuku:** None. **B. Gebelein:** None. **K. Campbell:** None.

Poster

660. Molecular Mechanisms of Neuronal Identity

Location: Hall A

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Program#/Poster#: 660.01/A29

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant AA013440

Texas A&M Womens Health in Neuroscience Program

Title: miRNA-pseudogenes interactions as a regulator of neural stem cell pluripotency and a target for ethanol teratogenesis

Authors: N. SALEM, S. BALARAMAN, R. HOLGATE, E. RAYMOND, *R. C. MIRANDA; Neurosci. & Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr, Col. of Med., Bryan, TX

Abstract: Fetal alcohol exposure is a leading non-genetic cause of neurodevelopmental disability. Neural stem cells (NSCs) that give rise to most neurons of the adult brain during the

first and second trimester are particularly vulnerable. We previously found that ethanol exposure did not result in NSC death, but rather, the loss of NSCs due to premature maturation. Moreover, we found that a class of small non-protein-coding regulatory microRNAs (miRNAs) was decreased following ethanol exposure. We recently found that the loss of miRNAs result in expression of a network of genes that support premature NSC maturation. However, the question that remains is whether ethanol also specifically prevents NSC renewal by interfering with miRNA-regulated processes. To address this question, we assessed the regulation of the homeobox transcription factor, Oct4/POU5F1, which is important for maintaining stem cell renewal and pluripotency. The Oct4 family includes a number of transcribed non-protein coding pseudogenes. We hypothesized that these pseudogenes serve as miRNA sponges. Immuno-precipitation studies with the miRNA binding protein, Ago-2, showed that several Oct4 pseudogenes bind miRNAs in both the nucleus and cytoplasm of NSCs, supporting their role as miRNA sponges. Ethanol exposure resulted in a decrease in expression of Ago2-binding pseudogene transcripts and also decreased expression of Oct4 mRNA and protein. These data suggest that pseudogenes-miRNAs interaction may protect pluripotency factors and facilitate NSC renewal. These data also advance a novel mechanism for ethanol teratology in that ethanol exposure may disrupt long non-coding RNA (lncRNA)-mediated protection resulting in miRNA-mediated loss of renewal capacity in fetal NSCs.

Disclosures: N. Salem: None. **S. Balaraman:** None. **R. Holgate:** None. **E. Raymond:** None. **R.C. Miranda:** None.

Poster

660. Molecular Mechanisms of Neuronal Identity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 660.02/A30

Topic: A.01. Neurogenesis and Gliogenesis

Support: HD09402

Title: A role for NOTCH pathway in the specification of glial precursors during hindbrain development

Authors: *M. S. DOMOWICZ, M. MCGOVERN, J. G. HENRY, D. SANTANA, D. VAVAL, N. B. SCHWARTZ;
Pediatrics, Univ. of Chicago, Chicago, IL

Abstract: After neurogenesis in the developing brain, radial glial cells (RGCs) have the potential to differentiate into the astrocytes or oligodendrocytes, but our knowledge of the signaling pathways governing this decision *in vivo* are limited. We study the influence of the NOTCH pathway in regulating the gliogenic fate of RGCs in an embryonic-day-8 chick hindbrain slice culture preparation in which astrocytes differentiate and migrate in a manner similar to *in vivo* timing and patterning. Inhibition of the NOTCH pathway with inhibitors of γ-secretase, DAPT or RO4929097, induces up-regulation of the oligodendrocyte marker proteolipid protein (PLP) mRNA in areas where the astrocyte precursors migrate, suggesting a switch from astrocytic to oligodendrocytic fate. In order to confirm this possibility, we analyzed a battery of astrocyte and oligodendrocyte markers by *in situ* hybridization and real-time qPCR and followed the morphological changes of RGCs over time after DAPT treatment. The level and distribution of SOX2, a marker of stem cells was not altered by inhibition of the NOTCH pathway. mRNA levels for GFAP, a marker of mature astrocytes, SOX9 and aggrecan, markers of astrocyte precursors, decreased in a DAPT-concentration-dependent manner. Using qPCR, the oligodendrocyte precursors markers PDGFRα and OLIG2, as well as MBP, a marker of mature oligodendrocytes, were up-regulated by DAPT treatment. Furthermore, after following the migration and morphology changes of RGCs labeled by transient transfection with a green fluorescent protein vector, an increase in ectopically localized oligodendrocyte-like cells in cultures treated with DAPT was observed. Taken together, these results indicate that NOTCH activation is required for RGCs commitment to the astrocytic fate and that inhibition of the NOTCH pathway in RGCs change their commitment towards the oligodendrocyte fate.

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Poster

660. Molecular Mechanisms of Neuronal Identity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 660.03/A31

Topic: A.01. Neurogenesis and Gliogenesis

Support: CONACYT 127357

Title: Gas1 is present in the germinal niches of the developing dentate gyrus and cortex

Authors: *E. ESTUDILLO, P. ZAVALA, G. PEREZ-SANCHEZ, A. AYALA-SARMIENTO, J. SEGOVIA-VILA;
CINVESTAV, MEXICO CITY, Mexico

Abstract: Gas1 is a pleiotropic protein that inhibits tumor growth when overexpressed but during development acts as a co-receptor for sonic hedgehog to promote proliferation and survival of different developing organs and systems. Gas1 has been extensively studied during development in the cerebellum, however in other structures of the central nervous system the information is limited to *in situ* hybridization studies. We provide information about the pattern expression of Gas1 during different developmental stages of the cortex and dentate gyrus of the mouse. The levels of Gas1 decrease in the developing brain as mice grow and it is mainly in progenitor cells during the development of cortex and the dentate gyrus.

Disclosures: **E. Estudillo:** None. **P. Zavala:** None. **G. Perez-sanchez:** None. **A. Ayala-sarmiento:** None. **J. Segovia-vila:** None.

Poster

660. Molecular Mechanisms of Neuronal Identity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 660.04/A32

Topic: A.01. Neurogenesis and Gliogenesis

Support: CIHR-10002944

Title: Neurog2 and Ascl1 play a key role in development of mouse ventromedial hypothalamus

Authors: *S. ASLAN POUR KAL BOLANDI[1], G. WILKINSON[2], C. SCHUURMANS[2], D. M. KURRASCH[1];
[1]Neurosci., [2]Biochem. & Mol. Biol., Univ. of Calgary, Calgary, AB, Canada

Abstract: The hypothalamus is a small but powerful region of the brain that plays a key role in maintaining overall physiological homoeostasis. Despite this important role, the molecular programs that drive hypothalamic development are just starting to be explored. The ventromedial hypothalamus (VMH) is a hypothalamic nucleus important for controlling satiety and reproductive behaviours, and has been shown previously to require the proneural gene *Neurogenin 3* for proper neuronal terminal differentiation. Here, we asked whether other proneural genes, namely *Achaete-scute homolog1* (*Ascl1)* and *Neurogenin2 (Neurog2)* might also play a key role in VMH development, given their importance in specifying cortical neurons and that they are also expressed in hypothalamic progenitors. To investigate more fully the expression pattern of these genes in the embryonic hypothalamus, we conducted *In situ* Hybridization (ISH) assays on the brains of wild type (WT) mice at different embryonic stages from e10.5 to P0, a period that encompasses VMH development. Our results revealed that *Ascl1*

and *Neurog2* have distinct spatiotemporal expression profiles, suggesting that different proneural genes might play a role in establishing hypothalamic neuronal identities during development. Next, using *Neurog2*$^{-/-}$ and *Ascl1*$^{-/-}$ mice, we investigated whether loss of *Neurog2* and *Ascl1* affects VMH cell fate decisions. Specifically we immunostained *Neurog2*$^{-/-}$ and *Ascl1*$^{-/-}$ brain slices during VMH neuronal migration (e.g., e15.5-e16.5) and in the mature VMH nucleus (e17.5-P0). Our results demonstrate that while *Neurog2*-null mice at e15.5 lose cell-specific markers within the central and ventrolateral domains of the VMH nuclear structure, *Ascl1*-null mice at e16.5 lose marker expression complementarily within the dorsomedial and some of the central domains. In both *Neurog2*- and *Ascl1*-null neonatal (P0) animals, however, a significant decrease in overall VMH-positive neurons is observed throughout the entire nucleus regardless of subdomain localization. Lastly in *Neurog2*$^{-/-}$*;Ascl1*$^{-/-}$ double knock out mice at e17.5, expression of VMH markers in both the dorsomedial and central domains were lost while ventrolateral markers domain showed only a slight decrease in the number of VMH-positive cells. This phenotype was much severe compared to single knock out at e17.5. In conclusion, here we showed that *Neurog2* and *Ascl1* are necessary for proper VMH development.

Disclosures: S. Aslan Pour Kal Bolandi: None. **G. Wilkinson:** None. **C. Schuurmans:** None. **D.M. Kurrasch:** None.

Poster

660. Molecular Mechanisms of Neuronal Identity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Grants-in-Aid for Young Scientists (B)

Grants-in-Aid for Scientific Research (C)

research grants from the Mitsubishi Foundation

Title: Increased dosage of DYRK1A enhances STAT activity and astrocytic differentiation of neocortical progenitors in a mouse model of Down's syndrome

Authors: *N. KURABAYASHI, K. SANADA;
The Univ. of Tokyo, Tokyo, Japan

Abstract: Down's syndrome (DS) occurs in approximately 1 in 700 live births and is caused by trisomy for human chromosome 21. The disorder is characterized by abnormalities in

neurological, skeletal, cardiovascular and immunological systems. Particularly, individuals with DS commonly exhibit mental retardation, which is associated with anomalies in brain development such as reduced neuronal production and increased astrocyte generation. Accumulating evidence suggests that differentiation of neural progenitors is deregulated in DS brains, and the deregulation contributes to brain developmental defect. Nevertheless, the molecular basis underlying the deregulation of progenitor cell fate decisions, particularly enhanced production of astrocytes in DS, is poorly understood. In this study, we demonstrate that differentiation of neocortical progenitors into astrocytes is enhanced by DYRK1A, a Ser/Thr kinase encoded on human chromosome 21. In the Ts1Cje mouse model of DS, overexpression of DYRK1A augments the propensity of progenitors to differentiate into astrocytes. We also found that increased dosage of DYRK1A is linked to deregulation of STAT, a transcription factor critical for astrogliogenesis. Overexpression of DYRK1A in wild-type progenitors increases STAT3 phosphorylation at Ser727, a regulatory site that enhances STAT3 activity. In addition, the STAT transcriptional activity is elevated upon increased dosage of DYRK1A in progenitors. On the other hand, STAT3 Ser727 phosphorylation and STAT activity are elevated in Ts1Cje progenitors and downregulating DYRK1A expression attenuates the deregulation of STAT. In sum, our work indicate that potentiation of the DYRK1A-STAT pathway in progenitors contributes to aberrant astrogliogenesis in DS.

Disclosures: **N. Kurabayashi:** None. **K. Sanada:** None.

Poster

660. Molecular Mechanisms of Neuronal Identity

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant MH083804

NIH Grant MH070596

Title: An assessment of the requirements for the Fgf receptor substrate (FRS) genes during early telencephalon development

Authors: ***S. NANDI**[1], G. GUTIN[1], N. KAMATKAR[1], K. W. LEE[2], F. WANG[3], G. FISHELL[4], M. GOLDFARB[2], J. M. HEBERT[1];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Hunter Col., NY, NY; [3]Texas A&M Hlth. Sci. Ctr., Houston, TX; [4]NYU Langone Med. Ctr., NY, NY

Abstract: FGF signaling is critical for forebrain development. Two FGF receptor substrate (FRS) proteins, FRS2 and FRS3, were proposed to play important roles in directly tranducing signal from the FGF receptors. However, their specific contributions in mediating FGF-dependent processes during early telencephalon development are unknown. Here, using a loss of function (LOF) approach we demonstrate that Frs3 is dispensable and that FRS2 protein is compensatorily upregulated in embryonic telencephalon of Frs3 mutants. In contrast, Frs3 is unable to fully compensate for the loss of Frs2 function during early telencephalon development. Moreover, we observe that Frs2 and Frs3 are together required for activation of MAPK and CREB1 in neural stem/progenitor cells and for differentiation of MGE neurons. We also propose that the Frs genes in contrast to their roles in stem/progenitor cells, may play an inhibitory role in CREB1 activation in the earliest-born neurons in a MAPK-dependent or -independent manner. Our LOF studies involving Fgf receptor 1 (Fgfr1) and Frs genes, together with analyses of Fgfr1 mutants with a deletion in the FRS-binding site of the FGFR1 further highlight a role for FRS proteins as primary if not the only modules of signal transduction downstream of FGFR1 during early telencephalon development. Thus our study demonstrates essential roles for FRS proteins relevant to FGF signaling *in vivo*. Since Foxg1-Cre-driven loss of three Fgfr (Fgfr1, Fgfr2 & Fgfr3) genes leads to a complete absence of telencephalon and that telencephalon although reduced in size is still present in the Frs2 and Frs3 double mutant, raise the possibility that FGF signaling in early telencephalon is mediated by non-FRS dependent mechanisms primarily involving FGFR2 & FGFR3.

Disclosures: **S. Nandi:** None. **G. Gutin:** None. **N. Kamatkar:** None. **K.W. Lee:** None. **F. Wang:** None. **G. Fishell:** None. **M. Goldfarb:** None. **J.M. Hebert:** None.

Poster

660. Molecular Mechanisms of Neuronal Identity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Boettcher Foundation Webb-Waring Biomedical Research Award

DoD W81XWH-14-1-0566

Title: Identification of gene regulatory networks in cone development

Authors: *T. ELISEEVA[1], J. A. BRZEZINSKI[1], K. JONES[2], K. PARK[1];
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Abstract: Humans depend on their color-sensitive cone photoreceptors for high visual acuity. Loss of these photoreceptors in disease can result in blindness. It has been difficult to study cone development because they make up a small percentage of retinal cells in rodent and primate models and form asynchronously over an extended period. Treatment of cultured embryonic (E) day 14.5 mouse retinas with the Notch inhibitor DAPT causes progenitors to synchronously form a cone-dominant retina by two days of treatment. To identify the genes responsible for cone development, we used RNA-seq to compare the transcriptomes of DAPT-treated retinas with their control counterparts at several time-points between 6 and 48 hours of treatment. Our setup yielded over 20 million yields per sample on average. Statistical analysis reveals hundreds of differentially expressed genes between the treatment groups and how they change over time as the retinal cells become more cone-like. This dataset provides a valuable tool for studying the gene regulatory events that govern cone development. In addition, this technique will reveal early cone-specific markers that will help us evaluate early fate choice events in cone genesis, which are largely unknown.

Disclosures: **T. Eliseeva:** None. **J.A. Brzezinski:** None. **K. Jones:** None. **K. Park:** None.

Poster

660. Molecular Mechanisms of Neuronal Identity

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH K99/R00 NS072192

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NIH R01 DC011534

Pediatric Hydrocephalus Foundation

Eleanor and Miles Shore Fellowship Program for Scholars in Medicine/Boston Children's Hospital Career Development Award

Title: Progressive differentiation and instructive capacities of amniotic fluid and cerebrospinal fluid proteomes following neural tube closure

Authors: *K. CHAU[1,2], M. SPRINGEL[1,2], K. BROADBELT[1], H.-Y. PARK[1], S. TOPAL[1,3], M. LUN[4,1], H. MULLAN[1], T. MAYNARD[5], H. STEEN[1], A. LAMANTIA[5], M. LEHTINEN[1,2]; [1]Boston Children's Hosp., Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]UMass Med. Sch., Worcester, MA; [4]Boston Univ. Sch. of Med., Boston, MA; [5]The George Washington Univ. Sch. of Med. and Hlth. Sci., Washington DC, DC

Abstract: After neural tube closure, amniotic fluid (AF) captured inside the neural tube forms the nascent cerebrospinal fluid (CSF). Stem cells of the neural plate contact CSF-filled ventricles, proliferate and differentiate to form the mammalian brain. Using *in vivo* ultrasound imaging, we quantified the dynamic expansion of the ventricular-CSF space from its time of inception. We then developed tools to obtain pure AF and nascent CSF, before and after neural tube closure. Using quantitative proteomics, we define how the AF and CSF proteomes diverge during the course of mouse development. Using embryonic neural explants, we demonstrate that age-matched fluids promote Sox2-positive neurogenic identity in the developing forebrain and olfactory epithelia. Nascent CSF also stimulates Sox2-positive self-renewal of forebrain progenitor cells, akin to LIF-mediated signaling. Taken together, our comprehensive proteomic resource should enable new approaches for the investigation of fluid-tissue interactions during this understudied, yet highly vulnerable stage of early brain development.

Disclosures: K. Chau: None. **M. Springel:** None. **K. Broadbelt:** None. **H. Park:** None. **S. Topal:** None. **M. Lun:** None. **H. Mullan:** None. **T. Maynard:** None. **H. Steen:** None. **A. LaMantia:** None. **M. Lehtinen:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.01/A37

Topic: A.01. Neurogenesis and Gliogenesis

Title: Autism-associated proteins Negr1 and FGFR2 together regulate cell migration and autism-related behaviors in mice

Authors: *J. SZCZURKOWSKA[1], F. PISCHEDDA[2], B. PINTO[4,1], F. MANAGO[1], C. HAAS[5], F. PAPALEO[1], M. SCHÄFER[6], G. PICCOLI[3], L. CANCEDDA[1];
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Abstract: The mammalian cerebral cortex is a remarkably complex structure, and establishment of cortical neural circuitries requires its unique laminar organization during neuronal migration. Accordingly, disruption in neural migration can lead to brain malformations with functional consequences on proper wiring of the neuronal network, as already described in neurodevelopmental disorders such as Autism Spectrum Disorders (ASD). Common knowledge indicates cell-adhesion molecules (CAMs) as essential for proper neural migration. Neuronal growth regulator 1 (Negr1) is a cell adhesion molecule, and NEGR1 gene mutations have been recently associated to autism spectrum disorders (ASD). So far, nothing is known about Negr1 function in *in vivo* neurodevelopment. By *in utero* electroporation coupled with RNA interference (siRNA), we downregulated Negr1 expression in late-born pyramidal neurons migrating to the superficial layers of the neocortex. We found that Negr1 siRNA caused ectopic positioning of neurons concentrated at the border between layer 5 and layer 4 in the somatosensory cortex. Downregulation of Negr1 did not cause migration defects in the motor or prefrontal cortices. Moreover, we found that FGFR2 and its partner NCAM physically interact with Negr1 to activate ERK signaling. Interestingly, downregulation of FGFR2 and NCAM *in utero* resulted in a strikingly similar phenotype on neuronal migration, as found for Negr1, but defective morphological maturation presented different characteristics in Negr1, FGFR2 or NCAM downregulated neurons. Accordingly, overexpression of FGFR2 as well as pharmacological activation of ERK signaling rescued the effect Negr1-downregulation on migration, but not on morphological maturation. In agreement with association of Negr1, FGFR2 and ERK signaling to autism, we found that downregulation of Negr1 or FGFR2 in the embryonic somatosensory cortex resulted in decreased number and complexity of ultrasound vocalizations in pups and that overexpression of FGFR2 as well as pharmacological activation of ERK signaling rescued the ultrasonic vocalization upon Negr1 downregulation. Finally, we further confirmed the above findings in Negr1 knock out mice, which showed defective cortical layering and reduced ultrasonic vocalizations. These data suggest that Negr1/FGFR2 complex is necessary for proper neuronal migration of pyramidal neurons in the somatosensory cortex and indicate a possible role for defective Negr1/FGFR2/ERK signaling and related migration impairment in autism.

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

Poster

661. Cell Migration in Neurodevelopment

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American Health Assistance Foundation

Consortium for Frontotemporal Dementia Research

Bright Focus Foundation

Lupe Murchison Foundation

The Ted Nash Long Life Foundation

Title: Reelin, ephs and ephrins: neuronal migration or synaptic plasticity?

Authors: *T. POHLKAMP[1], X. XIAN[1], L. XIAO[2], R. SULTANA[2], H. H. BOCK[6], M. HENKEMEYER[2], J. HERZ[1,3,4,5];
[1]Mol. Genet., [2]Dept. of Developmental Biol., [3]Ctr. for Translational Neurodegeneration Res., [4]Neurosci., [5]Neurol. and Neurotherapeutics, Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [6]Clin. for Gastroenterology, Hepatology and Infectiology, Heinrich-Heine-University, Duesseldorf, Germany

Abstract: The greatest risk factor for Alzheimer's Disease (AD) today is advancing age and as life expectancy increases so does the number of new cases. The pathogenesis of AD is not well understood, nor is there a cure or effective treatment available. There is increasing evidence that the extracellular signaling protein Reelin as well as the cell-cell interaction receptors EphB/ephrin-B play important roles in the onset of AD. In addition to its important function in cortical layer formation during brain development, in the adult brain, Reelin by binding to lipoprotein receptors modulates synaptic functions and delays Aβ deposition, the hallmark feature of AD. Also EphB/ephrin-B signaling is protective against Aβ fibril formation and like Reelin acts on NMDA receptor signaling. Recently our lab and others found that Reelin can signal through a common complex containing EphB/ephrin-B and ApoE receptors (Senturk et al., 2011, Nature; Bouche et al., 2013, Cell Res), but the function of their interplay remains unclear. In contrast to published data (Senturk et al., 2011) we show that this interplay is not effective for radial pyramidal neuron migration during neocortical development. By using several techniques (NeuN, TBR1, and BRN1 immunoreactivity, Nissl, DAPI staining,

distribution of Thy1-GFP neurons) we examined knockout mice deficient in all three ephrin-B ligands (Efnb1;2;3-/-) or three EphB receptors (Ephb1;2;3-/-) as well as Ephb2-/-;Reln+/- and Efnb3-/-;Reln+/- compound mutant mice for layer malformation. We could not confirm the reported overmigration phenotype or any striking cortical layer malformations for Efnb1;2;3-/- and Efnb3-/-;Reln+/-. Reelin signaling induces Dab1 turnover, consequently in reeler mice Dab1 levels are elevated, which was not true for any of the mutants we analyzed, and serves as evidence that ephrin-Bs are not essential for canonical Reelin signaling. Understanding the interaction between Reelin and EphB/ephrin-B signaling will innovate targeted strategies to combat AD. In future studies we aim to understand how these signaling molecules interact in the complex machinery that controls synaptic function and how they influence the aging brain.

Disclosures: T. Pohlkamp: None. **X. Xian:** None. **L. Xiao:** None. **R. Sultana:** None. **H.H. Bock:** None. **M. Henkemeyer:** None. **J. Herz:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.03/A39

Topic: A.01. Neurogenesis and Gliogenesis

Support: Swiss National Foundation

Special Program University Medicine (SPUM)

Title: Wnt signaling regulates multipolar-to-bipolar transition of migrating neurons in the cerebral cortex

Authors: *R. BOCCHI, M. BOITARD, K. EGERVARI, V. PETRENKO, B. VIALE, S. GREMAUD, E. ZGRAGGEN, P. SALMON, J. Z. KISS;
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Abstract: Radial migration of newborn excitatory neurons in the developing neocortex is a complex multistep process that is precisely regulated in space and time. It includes multipolar migration, multipolar-to-bipolar transition, radial glia-guided locomotion and terminal somal translocation. Increasing evidence suggests that different transition steps are regulated by distinct signaling pathways and mutations as well as deregulation of these molecular pathways could result in migration disorders. While it has been speculated that Wnt signaling might control neuronal migration, its direct role remains to be established. Using video time-lapse imaging and

reporter constructs for Wnt canonical and non-canonical signaling cascades, we now show that dynamically regulated activity states of canonical Wnt/ß-catenin as well as non-canonical signaling during specific migratory phases are crucial for proper polarization and migration of late generated pyramidal neurons. Transient down-regulation of canonical Wnt/ß-catenin signaling activity appears to be required for the proper transition of pyramidal precursors from multipolar into bipolar state. We found that time-dependent regulation of Wnt5A-mediated non-canonical signaling reduces canonical Wnt signaling in a cell-autonomous manner during the multipolar-to-bipolar transition. Down-regulation of canonical Wnt signaling by Wnt5A allows cells to express ephrin-B1, thereby enabling polarization and initiation of glia-guided locomotion. These findings highlight the role of a crosstalk between the Wnt5A/non-canonical and Wnt/ß-catenin signaling pathways in regulating pyramidal cell migration via multipolar-to-bipolar transition.

Disclosures: **R. Bocchi:** None. **M. Boitard:** None. **K. Egervari:** None. **V. Petrenko:** None. **B. Viale:** None. **S. Gremaud:** None. **E. Zgraggen:** None. **P. Salmon:** None. **J.Z. Kiss:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.04/A40

Topic: A.01. Neurogenesis and Gliogenesis

Title: Spontaneous neurodevelopmental malformations in rats and mice are visible with magnetic resonance histology

Authors: *R. L. RAMOS;
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Abstract: Several strains of mice and rats exhibit spontaneous neurodevelopmental malformations of the neocortex and/or cerebellum due to deficits in neuronal migration during pre- and postnatal periods, respectively. Several factors complicate the study of the development, anatomy, and physiology of malformations including: 1] malformations are not identifiable a priori in the living animal or *in situ*, 2] histological conformation of malformations is time- and labor-intensive, 3] histological analyses introduce limitations to quantitative descriptions of malformations such as a poor estimates of size, volume, spatial and areal extent, and 3D morphology. Therefore, methods to identify malformations *in vivo* or *in situ* would open new avenues to research associated with malformations. Using archival imaging data from the Duke University Center for *In vivo* Microscopy (an NIH/NIBIB Biomedical Technology Resource

Center - P41 EB015897), I will demonstrate that spontaneous neurodevelopmental malformations are visible in both mice and rats *in situ* with magnetic resonance histology. Moreover, several imaging modalities were found to be compatible for identifying malformations including T1, T2, and diffusion tensor imaging. The implications of these data will be discussed as well as the possible applications of magnetic resonance histology in the study of brain development and neuronal migration disorders in mouse models.

Disclosures: R.L. Ramos: None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.05/A41

Topic: A.01. Neurogenesis and Gliogenesis

Support: KRF 2640044

Title: Sp9 regulates MGE-derived cortical interneuron development

Authors: *Z. LIU, Y. YOU, Q. LIANG, Z. YANG;
Fudan Univ., Shanghai, China

Abstract: Interneurons in mammalian cortical structures (neocortex, hippocampus and olfactory bulb) are mainly generated from subcortical ganglionic eminence including medial, lateral and caudal ganglionic eminence (MGE, LGE and CGE). Here we explore the roles of the zinc finger transcription factor Sp9 in regulating neocortical interneuron development. We show that Sp9 is widely expressed in the MGE, LGE and CGE progenitors and postmitotic cells. Fate mapping studies using Sp9-Cre knockin mice suggest that virtually all cortical GABAergic interneurons are derived from Sp9-expressing cells. In Sp9 mutant embryos, MGE-derived interneurons tangentially migrate to the cortex, but exhibit defects in populating the marginal zone and superficial parts of the cortical plate. In P21 Sp9 mutant cortex, ~50% of MGE-derived cortical interneurons are lost, whereas CGE-derived cortical interneurons are mildly increased. RNA-Seq and ChIP-Seq experiments provide evidences that Sp9 mediates these effects in the MGE mainly through promoting expression of Lhx6 that regulates interneuron migration and through inhibiting the expression of Sox6 that is also controlling cortical interneuron development.

Disclosures: Z. Liu: None. **Y. You:** None. **Q. Liang:** None. **Z. Yang:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.06/A42

Topic: A.01. Neurogenesis and Gliogenesis

Title: Injectable biomaterials alter progenitor cell migration and inflammation after injury to the young adult brain

Authors: *R. MOTALLEB[1], E. J. BERNS[2], S. I. STUPP[3], G. H. KUHN[1];
[1]Neurosci. and Physiol., Goeteborg, Sweden; [2]Dept. of Biomed. Engin., Chicago, IL; [3]Dept. of Materials Sci. and Engineering. Dept. of Chemistry. Dept. of Med. and Inst. for BioNanotechnology in Med., Chicago, IL

Abstract: The regenerative capacity of the adult brain is insufficient when stroke or brain trauma occurs even though there is a proliferating population of neural stem cells capable of regenerating damaged tissue. However, the combination of stem cell research and biomaterials opens up new possibilities for regenerative medicine. Endogenous neuroblasts migrate from the subventricular zone along the rostral migratory stream (RMS) to the olfactory bulb, but as insult occurs to the brain, these cells can change path and migrate to the injured area, although not to the extent needed. By redirecting these cells we hope to increase the population of neural stem cells that reach the affected area and hence repopulate and regenerate the damaged tissue. Here we utilize a self-assembling peptide amphiphile (PA) with a Tenascin-C epitope to redirect the migration of endogenous neuroblasts from the rostral migratory stream to the cortex. Stereotactic injections of PA were performed in the RMS of young adult rats as the syringe is retracted, creating a tract of PA to the cortex. The PA material will polymerize forming nanotubular structure upon injection into the brain due to contact with Ca2+. PA is biodegradable lasting up to 4 weeks *in vivo*. Preliminary results show that the peptide amphiphile does not elicit an increased astrocytes or microglia response compared to the saline/vehicle injected controls. Neuroblasts (DCX+) are able to infiltrate the biomaterial and migrate to the cortex one week post-injection. Our results indicate that migrating neuroblasts can be redirected using an injectable biomaterial, which in turn can increase the number of neuroblasts reaching the damaged tissue. Injury also elicits an immune response resulting in scar tissue and inflammation, therefore we analyzed the effect of PA with the fibronectin-derived epitope RGDS on neuroinflammation using the same setup as previously described. Immunostaining shows significantly lower cell density for GFAP+ and Iba-1+ cells surrounding the needle tract after RGDS PA injection compared to vehicle. Intensity measurements using GFAP, Iba-1 and CD68

also show a significantly lower activation of astrocytes and microglia/macrophages up to 100 μm bordering the lesion site compared to vehicle, indicating a reduced glial scar and a smaller inflammatory response.

Disclosures: R. Motalleb: None. **E.J. Berns:** None. **S.I. Stupp:** None. **G.H. Kuhn:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.07/A43

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIMH Grant R01 MH097949-01

Autism Speaks Pilot Grant#7359

Title: Rapamycin can prevent, but not reverse, aberrant migration of pten knockout neurons

Authors: *S. A. GETZ[1], T. DESPENZA, Jr[2], M. LI[1], B. W. LUIKART[1];
[1]Geisel Sch. of Med. At Dartmouth, Lebanon, NH; [2]Icahn Sch. of Med. at Mount Sinai, New York City, NY

Abstract: The PI3K/Akt/mTor pathway is important in cell growth, survival and proliferation. Phosphatase and tensin homolog (PTEN) is a major negative regulator of that pathway through the dephosphorylation step of PIP3 into PIP2. *PTEN* mutations have been found in a subset of individuals with Autism and macrocephaly. To examine whether Pten knockout (KO) in granule neurons alters their migration, fluorescent protein linked cre- and non cre-expressing retroviruses were co-injected into the hippocampal dentate gyrus of *Pten* $^{Flox/Flox}$ mice at postnatal day 7. Animals were sacrificed at various days post-injection (DPI) to reveal Pten KO granule cell neurons migrate significantly further from the hilus compared to wild-type controls beginning at 12.5 DPI and remaining that way every timepoint thereafter. To test whether the migration phenotype could be prevented or reversed, we administered daily intraperitoneal injections (IP) of Rapamycin, an mTorC1 inhibitor. The preventative group received Rapamycin from 3-14 DPI, when the enhanced migratory phenotype of Pten KO neurons is being established. The reversal group received Rapamycin from 14-24 DPI, after the enhanced migratory phenotype has been established. Soma size and migration analysis reveals that Rapamycin can prevent and reverse somal hypertrophy seen in Pten KO neurons, but it can only prevent but not reverse the

enhanced migration of Pten KO neurons. This may have implications for the clinical use of Rapamycin in treating *PTEN*-mutation associated diseases.

Disclosures: **S.A. Getz:** None. **T. DeSpenza:** None. **M. Li:** None. **B.W. Luikart:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.08/A44

Topic: A.01. Neurogenesis and Gliogenesis

Support: CNpQ / Brazil

Title: Embryonic BAGAergic system is affected by alcoholism in rats

Authors: *D. M. SANTOS[1], R. N. ISAYAMA[1], R. R. RAMOS[1], E. N. YAMAZAKI[2], D. UZIEL[2];
[1]UNICASTELO, Fernandopolis, Brazil; [2]UFRJ, Rio de Janeiro, Brazil

Abstract: In this study we aimed to investigate the effect of ethanol exposure on the development of the GABAergic system in the telencephalon. We administered ethanol (2g/Kg) using a gavages cannula to pregnant swiss mice between E11 and E14. At E13, we collected blood to measure plasmatic concentrations at specific time points after ethanol gavages. At E14, animals were sacrificed, embryos removed and dissected. Brains were used for immunohistochemistry (GABA), western Blot (GAD65/67), or labeling for TUNEL. Some litters were kept after delivery and tested in the open field. Our results indicated that serum blood reached a pick (120±10mg/dl) after 1h and decreased until 3h. Roughly we did not identify macroscopic modifications, such as excencephaly or anencephaly. Quantification of the immunohistochemistry for GABA showed a decrease manly at the lateral ganglionic eminence (LGE), but no significant alterations in the medial ganglionic eminence (MGE) or in the developing cortical plate. We also found a significant increase in the GAD65/67 expression at the LGE but not in the MGE or in the cortex. In contrast, TUNEL labeling revealed a significant raise in the rate of neuronal death in the cortex and in the hippocampal anlagen. In the behavioral test no modification in animal's exploratory behavior was identified. We conclude that 2g/Kg ethanol during a short period at this specific developmental lag does not lead to significant alterations in the cortical GABAergic system, but leads to mild increase in GABA and GAD expression in the proliferative zones of the basal telencephalon and to cell death in the dorsal telencephalon.

Disclosures: **D.M. Santos:** None. **R.N. Isayama:** None. **R.R. Ramos:** None. **E.N. Yamazaki:** None. **D. Uziel:** None.

Poster

661. Cell Migration in Neurodevelopment

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.09/A45

Topic: A.01. Neurogenesis and Gliogenesis

Support: CONACyT Scholarships number 244955

Title: Cell migration of pituitary adenoma cells on collagen type I-III

Authors: ***D. AVILA**[1], A. ORTIZ-PLATA[2], C. SOLANO-AGAMA[1], M.-E. MENDOZA-GARRIDO[1];
[1]Ctr. For Res. and Advanced Studies, Mexico City, Mexico; [2]Natl. Inst. of Neurol. and Neurosurg., Mexico City, Mexico

Abstract: During the tumoral progression the composition and biomechanical properties of the extracellular matrix (ECM) are modified, and it influence the cells behavior, for example regulating the cell migration. In the pituitary adenomas it has been observed an up-regulation of ECM proteins; a higher deposition of fibrillary type I collagen was observed, particularly in invasive macroadenomas. Little is known about the regulatory mechanism of cell migration in pituitary adenomas. It has been suggested that the remodeling of collagen fibers in the pituitary capsule are important factors in the infiltration of the cavernous sinus by pituitary adenoma cells. The objective is to characterize the cell migration pattern that pituitary adenoma cells present on a mixture of type I and type III collagen. Using a rat derived pituitary adenoma cell line (GH3 cell line) we analyze the cell migration pattern that adenoma cells adopt on collagen type I-III, in a two-dimensional system, employing a pharmacological approach we also analyzed the participation of the myosin light chain kinase (MLCK) and Rho-associated protein kinase (ROCK) in the arrangement of the actin cytoskeleton, in the localization of the myosin light chain (MLC) phosphorylation and finally in the regulation of cell migration. GH3 cells present a rounded-shape with a cortical actin belt arrangement with small blebs. MLCK inhibition blocked MLC phosphorylation at the cell substrate level but not at the cell periphery. MLCK-inhibited cells migrate 3.7 times more distance and 3.6 times faster, without showing persistence in the migration direction. Meanwhile, ROCK inhibition blocked the MLC phosphorylation at the cell periphery but not at the substrate level. ROCK-inhibited cells present an elongated-shape with a 0.5 times more spreading, and a decrement of 1.6 times the distance and the migration velocity.

In summary, our results suggest that these two kinases perform two different functions in cell migration, MLCK controls cell spreading and directionality in the movement and ROCK controls the rounded-shape and increases the net cell translocation.

Disclosures: D. Avila: None. **A. Ortiz-Plata:** None. **C. Solano-Agama:** None. **M. Mendoza-Garrido:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.10/A46

Topic: A.01. Neurogenesis and Gliogenesis

Title: ADP-ribosylation factor 6 controls the transition of migrating neurons from intermediate zone to cortical plate

Authors: *Y. HARA, H. SAKAGAMI;
Dept. of Anat., Kitasato Univ. Sch. of Med., Sagamihara-Shi, Japan

Abstract: Cortical layer formation in the cerebral cortex is one of the typical events in the mammalian brain. Neurons that are born in the ventricular zone (VZ) migrate to the pial surface with an inside-out manner along the fiber of radial glia. Recent studies revealed that transmembrane proteins such as N-cadherin, integrins, and connexins, regulate neuronal migration through cell-cell and/or cell-matrix interactions, and their expression on the plasma membrane is tightly regulated by vesicle trafficking factors that are involved in the process of secretion, endocytosis and recycling. Furthermore, some vesicle trafficking factors are identified as a responsible gene for periventricular heterotopia, and their dysfunction causes malformation of cortical layer. However, it largely remains unclear the mechanistic details of how vesicle trafficking factors regulate neuronal migration. In this study, we examined the expression and functional role of ADP-ribosylation factor 6 (Arf6), a critical regulator of endosomal trafficking, in the cortical layer formation. *In situ* hybridization analysis revealed that Arf6 mRNA was expressed in all layers including VZ, intermediate zone (IZ), and cortical plate (CP) in the dorsal pallium of embryonic cerebral cortex. Immunostaining analysis revealed that Arf6 appeared as puncta that partly colocalized with EEA1 and syntaxin12, markers for early and recycling endosomes, respectively, in migrating neurons. Knockdown (KD) of Arf6 by *in utero* electroporation revealed that Arf6 was required for transition from IZ to CP by controlling the recycling of N-cadherin in migrating neurons. Furthermore, mFIP3, a dual effector for both Arf6 and Rab11, also regulates neuronal migration, and knockdown of mFIP3 caused accumulation of

N-cadherin in perinuclear region of migrating neurons, as observed in Arf6 KD. These results implicate that N-cadherin recycling pathway through Arf6/mFIP3 regulates the transition from IZ to CP during the cortical layer formation.

Disclosures: Y. Hara: None. **H. Sakagami:** None.

Poster

661. Cell Migration in Neurodevelopment

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NINDS NS066071

Title: Conditional knockout of paxillin in the nervous system disrupts migrating neuron morphology and delays cortical lamination

Authors: *M. RASHID[1], J. BELMONT[2], D. CARPENTER[2], C. TURNER[3], E. OLSON[2]; [1]Dept. of Neurosci. and Physiol., [2]Neurosci. and Physiol., [3]Cell and Developmental Biol., SUNY Upstate Med. Univ., Syracuse, NY

Abstract: The paxillin family of focal adhesion adaptor proteins are known to regulate integrin-based cellular adhesion and migration, yet their roles in cortical development remain largely unknown. This is due, in part, to the early embryonic lethality of paxillin knockout mice. Two members of this family, paxillin and Hic-5, are expressed by neuronal precursors and differentiating neurons during early cortical development. Here, we characterize the cortical phenotypes of Hic-5 knockout mice and paxillin conditional knockout mice. Consistent with prior findings, we show that Hic-5 knockout mice are postnatal viable, fertile and have no overt abnormalities in cortical anatomy. In contrast, nestin-cre driven deletion of paxillin in the developing cortex produces a subtle cortical phenotype. While nestin-cre; paxillin$^{flox/flox}$ (paxillin cKO) mice are also postnatal viable and fertile, histological analyses of the neonatal cortex reveals a disruption in the lamination of Cux1^{+} upper-layer projection neurons compared to littermate controls. On postnatal day 1 (P1), Cux1^{+} neurons are normally localized to layer 2-4 in wildtype cortex, while significantly more Cux1^{+} neurons are found in ectopic deep positions in the paxillin cKO cortex (p=0.002). However, by P35 no difference is detected in the distribution of Cux1+ cortical neurons between mutant and control (p=0.68). To further characterize the P1 phenotype, cell-autonomous suppression and deletion of paxillin was performed using an *in utero* electroporation approach. Both shRNA-mediated suppression and cre-mediated deletion of

paxillin produced a migration delay and altered the morphology of migrating neurons. Compared to control, paxillin deficient migrating neurons have shorter leading processes and multiple swellings in their leading processes. This phenotype is remarkably similar to the reported phenotype produced by deletion of focal adhesion kinase (FAK), a critical signaling partner of paxillin. These results demonstrate that paxillin functions in a cell-autonomous manner to control the morphology of migrating neurons and the pace of cortical development.

Disclosures: M. Rashid: None. **J. Belmont:** None. **D. Carpenter:** None. **C. Turner:** None. **E. Olson:** None.

Poster

661. Cell Migration in Neurodevelopment

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R.A.P is a Royal Society University Research Fellow

Title: INM-like migration of newly born post-mitotic rod photoreceptors in the early post-natal mouse retinal neuroepithelium

Authors: *N. D. AGHAIZU[1], K. WARRE-CORNISH[2], M. R. ROBINSON[1], P. V. WALDRON[1], R. R. ALI[1], R. A. PEARSON[1];
[1]Dept. of Gene and Cell Therapy, UCL Inst. of Ophthalmology, London, United Kingdom;
[2]Basic & Clin. Neurosciences, Kings Col. London, London, United Kingdom

Abstract: Purpose: The developing retina is a pseudostratified neuroepithelium (NE) in which retinal progenitor cells (RPCs) span the radial tissue extent and mature stratification is yet to form. RPCs give rise to all major retinal cell types by mitosis. Cell cycle progression is

synchronised with the translocation of RPC nuclei within the radial cell extents, a process called interkinetic nuclear migration (INM). Nuclei are located at the apical edge of the NE during M-phase and nascent neurons must thus consequentially migrate radially to reach their designated layers. Rod cells eventually occupy a layer directly adjacent to the apical limit and it is often assumed, therefore, that there is limited requirement for migration. Nonetheless, in our preliminary investigations, post-mitotic rod precursor cells displayed marked motility during post-natal development. We therefore sought to determine the properties, kinetics, and mechanisms of this motility to help our understanding of retinal development. Methods: GFP under the control of the Nrl promoter labels post-mitotic rod photoreceptor precursor cells. The migration of GFP+ rod precursor cell bodies (RoPCBs) was assessed *ex vivo* by time-lapse 2-photon microscopy of live retinae from post-natal day 1, 3 and 7 Nrl.GFP+/+ mice and by software tracking (Imaris) of GFP+ rod precursor cell bodies. Results: RoPCBs were found dispersed within the NE, from directly adjacent to up to ~90 μm away from the apical limit. All observed cells extended a permanent apically attached process, but rarely extended a basal process. At all time points examined, RoPCBs underwent significant movement along the apico-basal axis. The majority of movements were stochastic with only limited net displacement. However, at least two further modes of migration, rapid apically directed jumps and slower basally directed migration were identified. Strikingly, RoPCBs were observed to oscillate between apical and basal positons using these three modes of migration with kinetics similar to INM. By means of pharmacology we demonstrated that migration was significantly impeded by inhibition of dynein I motor protein and microtubule dynamics, but not by inhibition of Myosin II motor protein. Conclusion: We report that the nucleus and surrounding RoPCBs engage in oscillatory apico-basal movements, highly reminiscent of the INM displayed by cortical neural progenitor cells. Similar to cortical neural progenitor cells but in contrast to lower vertebrate RPCs, these movements appear to be microtubule-dependent but Myosin II independent. To our knowledge, this is the first observation of an INM-like motility in a post-mitotic neuronal cell population.

Disclosures: **N.D. Aghaizu:** None. **K. Warre-Cornish:** None. **M.R. Robinson:** None. **P.V. Waldron:** None. **R.R. Ali:** None. **R.A. Pearson:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.13/A49

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH

Title: Deletion of Dcc results in mispositioning of spiral ganglion neurons during cochlear development

Authors: *Y. KIM, S.-Z. WANG, H. W. TAO, L. I. ZHANG;
USC, Los Angeles, CA

Abstract: The spiral ganglion neurons (SGNs) are the first neurons in the auditory pathway to fire action potentials to relay sound information up to the auditory cortex. These bipolar neurons send dendrites to innervate the base of hair cells in their cochlea and their axons to the cochlear nucleus through the auditory portion of eighth cranial nerves. Several axon guidance molecules have been reported responsible for the proper organization of SGN positioning and innervation patterns in the cochlea. Here, we show that disruption of Dcc results in mis-migrated SGNs towards the sensory epithelium. Specific patterns of positioning of mis-migrated SGNs were observed in different developmental time periods. Initial observation of mis-migrated SGNs at E16.5 suggests that it is not an initial migratory defect during delamination of SGNs but possibly a defect in rearrangement of SGNs during the convergent extension. Disruption of Dcc also caused SGNs to be mispositioned along the central auditory pathway towards the cochlear nucleus. In addition, a defect in fiber routing of SGNs in both peripheral and central sides has been observed. Our findings demonstrate that Dcc is required for the correct positioning of SGNs and routing of their processes during the auditory circuit development.

Disclosures: Y. Kim: None. **S. Wang:** None. **H.W. Tao:** None. **L.I. Zhang:** None.

Poster

661. Cell Migration in Neurodevelopment

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Swedish Research Council

Nicholson Exchange Program

Title: N-cadherin and Astrotactin interact to regulate granule cell migration in the developing cerebellum

Authors: *Z. HORN, M. E. HATTEN;
Lab. of Developmental Neurobio., Rockefeller Univ., New York, NY

Abstract: The formation of a laminated cortex in the mammalian brain depends on directed migration of neuronal precursors. The neural cadherin, N-cadherin (Cdh2), is crucial for early development of the embryo and mediates neuron-glia attachment during glial-guided migration in the cerebral cortex. The role of Cdh2 in the development of the cerebellum is however still unclear. In this study, we assessed whether Cdh2 interacts with other adhesion proteins known to be involved in granule cell (GC) migration and maturation in the cerebellum. We found that Cdh2 co-immunoprecipitates with the two known members of the astrotactin family, Astn1 and Astn2, which regulate glial-guided migration of cerebellar GCs. This interaction is dependent on the MACPF, Fibronectin type III and EGF-like domains of the astrotactin protein. Interestingly, immunolabeling of GC/glia co-cultures showed that Cdh2 and Astn1 co-localize to the neuron-glia junctions of migrating GCs. Moreover, adhesion assays using *Drosophila* S2 cells indicated that Cdh2 and Astn1 interact in a heterophilic manner. To provide a genetic analysis of the role of *Cdh2* in GC development, floxed *Cdh2* mice were crossed with *NeuroD1-Cre* mice to delete *Cdh2* in migrating GCs. Structural studies showed that the cerebellum of *Cdh2* conditional mutant mice at P5 - P7 was 15 - 20% smaller than in control littermates, and BrdU/EdU labeling methods revealed a marked reduction in GC migration. In addition, the expression of the Astn1 protein was mislocalized in the GCs lacking *Cdh2*. Interestingly, Bergmann glial fibers were significantly decreased and misaligned in the mutants, indicating that homophilic and/or heterophilic interactions of Cdh2 function in Bergmann glial development. Taken together, our results suggest that Cdh2 regulates glial-guided migration of cerebellar GCs and may promote neuron-glia attachment by interacting with Astn1.

Disclosures: Z. Horn: None. **M.E. Hatten:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.15/A51

Topic: A.01. Neurogenesis and Gliogenesis

Title: Slit/Robo signals maintain motor neurons inside the spinal cord by regulating the integrity of the basement membrane

Authors: *M. KIM, H. LEE, G. S. MASTICK;
Biol., Univ. of Nevada, Reno, Reno, NV

Abstract: Spinal motor neurons are clustered in a well-organized pattern within the ventral spinal cord, and project axons out through the basement membrane to innervate their peripheral targets. The retention of motor neuron cell bodies within the spinal cord is critical to form their functional circuits. However, little is known about how spinal motor axons can exit, but not motor neuron cell bodies. The secreted Slit proteins are major repellents produced by the floor plate, and their Robo1 and Robo2 receptors mediate Slit repulsion. Importantly, Slit2 and both Robo receptors are expressed by spinal motor neurons. We found that some motor neurons migrated outside the spinal cord in Slit1/2 or Robo1/2 double knockout mouse embryos. More specifically, tests of single Slit or Robo alleles showed that Slit2 and Robo2 are necessary for maintaining motor neurons inside the spinal cord. We sought to test how abnormal Slit/Robo signals lead to emigrant phenotype in the spinal cord. Many reports suggest that the laminin-containing basement membrane is a substrate for neuron migration as well as a boundary that constrains motor neurons on a proper migratory path. We tested a major basement membrane protein, Dystroglycan (DG), a glycoprotein linking the extracellular matrix and the cytoskeleton. In the Slit1/2 knockout spinal cord, expression of DG was discontinuous, forming large gaps in the basement membrane. These gaps were associated with the ventral sites where motor neurons ectopically emigrate, suggesting that the basement membrane is critically required to prevent aberrant motor neuron migration. The fact that Robo1/2 mutants phenocopied Slit1/2 mutants suggests that the neuroepithelium of the wall of the neural tube produces or maintains the basement membrane in a Slit-dependent manner. Together, the findings from the present study suggest that Slit/Robo signals are required to retain spinal motor neurons inside the neural tube by regulating the integrity of the basement membrane. Further research will be needed to address a molecular basis on how, when and where the guidance signals are required for keeping spinal motor neurons on a proper position.

Disclosures: **M. Kim:** None. **H. Lee:** None. **G.S. Mastick:** None.

Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.16/A52

Topic: A.01. Neurogenesis and Gliogenesis

Support: BFU2008-04156

0458/GERM/06-10891

PII1I09-0065-8194

Title: Radial and tangential migration of telencephalic somatostatin neurons originated from the mouse diagonal area

Authors: ***J. L. FERRAN**[1], N. MORALES-DELGADO[1], P. MERCHÁN[2], B. CASTRO-ROBLES[3], M. MARTINEZ-DE-LA-TORRE[1], C. DÍAZ[4], L. PUELLES[1];
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Abstract: The telencephalic subpallium is the source of various GABAergic interneuron cohorts that invade the pallium via tangential migration. Based on genoarchitectonic studies, the subpallium has been subdivided into four major domains: striatum, pallidum, diagonal area and preoptic area (Puelles et al., 2013; Allen Developing Mouse Brain Atlas) and a larger set of molecularly distinct progenitor areas (Flames et al., 2007). *In utero* fate mapping and genetic lineage tracing studies have suggested that each subpallial subdivision produces specific sorts of inhibitory interneurons, distinguished by differential peptidic content, which are distributed tangentially to pallial and subpallial target territories (e.g., olfactory bulb, isocortex, hippocampus, pallial and subpallial amygdala, striatum, pallidum, septum). In this report we map descriptively the early differentiation and apparent migratory dispersion of mouse subpallial somatostatin-expressing (Sst) cells from E10.5 onwards, comparing their topography with the expression patterns of the genes Dlx5, Gbx2, Lhx7-8, Nkx2.1, Nkx5.1 (Hmx3), and Shh, which variously label parts of the subpallium. Whereas some experimental results suggest that Sst cells are pallidal, our data reveal that many, if not most, telencephalic Sst cells derive from de diagonal area (Dg). Sst-positive cells initially only present at the embryonic Dg selectively populate radially the medial part of the bed nucleus striae terminalis (from paraseptal to amygdaloid regions) and part of the central amygdala; they also invade tangentially the striatum, while eschewing the globus pallidum and the preoptic area, and integrate within most cortical and nuclear pallial areas between E10.5 and E16.5.

Disclosures: **J.L. Ferran:** None. **N. Morales-Delgado:** None. **P. Merchán:** None. **B. Castro-Robles:** None. **M. Martinez-de-la-Torre:** None. **C. Díaz:** None. **L. Puelles:** None.

Poster

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Title: Impaired interneuron development after disruption of Foxg1 disruption

Authors: *W. SHEN[1], Y. YANG[2], Y. WEI[3];
[1]Institude of Life Sci. of Southeast Univ., Jiangsu, China; [2]institude of life science of southeast university, Nanjing, China; [3]Institude of life science of Southeast university, Nanjing, China

Abstract: Although interneurons play pivotal roles in the modulation of cortical function, the mechanisms that control interneuron development remain to be elucidated. This study aimed to explore a new role for Foxg1, a candidate gene for West syndrome and Rett syndrome, in interneuron development. By crossing Foxg1fl/fl mice with a Dlx5/6-Cre line, we determined that the conditional disruption of Foxg1 in the subpallium results in defects in interneuron differentiation and tangential migration. In developing interneurons, the expression levels of several receptors, including roundabout-1 (Robo1), Eph receptor A4 (EphA4) and (C-X-C motif) receptor 4/7 (Cxcr4/7), were strongly down-regulated, which led to migration defects after Foxg1 ablation. The transcription factors Dlx1/2 and Mash1, which have previously been reported to be involved in interneuron development, were significantly up-regulated at the mRNA levels. Notably, Prox1, which is a transcription factor that functions as a key regulator in the development of excitatory neurons, was also dramatically up-regulated at both the mRNA and protein levels. This result suggests that Prox1 is also important for interneuron development. Our work demonstrates that Foxg1 may act as a critical up-stream regulator of Dlx1/2, Mash1 and Prox1 to control interneuron development. These findings will improve our understanding of the molecular mechanisms of Foxg1-related diseases, including both West and Rett syndromes.

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Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Title: The essential role of RBM8a in brain development

Authors: *C. MCSWEENEY[1], D. REYNOLDS[2], A. SEBASTIAN[1], J. VITALE[1], Y. ZHOU[1], F. DONG[1], D. DENG[1], L. LIU[1], X. JIANG[1], J. JIANG[1], Y. WANG[3], I. ALBERT[1], Y. MAO[1]; [1]Penn State Univ., State College, PA; [2]Univ. of California Irvine, Irvine, CA; [3]Shanghai Jiaotong Univ., Shanghai, China

Abstract: Nonsense-mediated mRNA decay (NMD) is an RNA surveillance mechanism that ensures the degradation of mRNAs carrying premature termination codons (PTCs). This mechanism relies on several factors, which form a tetramer known as the exon junction complex (EJC). Mutations in multiple EJC factors have been reported to cause X-linked mental retardation and autism (Tarpey et al., 2009; Laumonnier et al., 2010; Addington et al., 2010). This strongly indicates a potential role for NMD in the pathogenesis of autism. Using the cre-loxp system, we selectively knocked out the protein RBM8a in neural stem cells. *Nes-Cre;RBM8a*$^{fl/+}$ mice presented with microcephaly (even after accounting for small body size), decreased body size, impaired mobility, and a life span of approximately 20 days. *Nes-Cre;RBM8a*$^{fl/+}$ mice have decreased cell density in the cortex, and throughout the CA regions of the hippocampus. The structural morphology of the hippocampus is also abnormal. The cortex of *Nes-Cre;RBM8a*$^{fl/+}$ mice is very thin, and the two hemispheres fail to meet on the midline. Interestingly, these mice also have disorganized cortices. Cux1, a marker for cortical layers 2/3 is present in cells all throughout the cortex of *Nes-Cre;RBM8a*$^{fl/+}$ mice, with the greatest density in layers 5/6. Foxp2, a marker for cortical layer 5/6, is also present in layer 4. This indicates either deficits in cell fate specification, or deficits in migration. Seeing as children with autism have been observed to have disorganized neocortices (using post mortem *in situ* hybridization), this phenotype further implicates RBM8a as a potential risk gene for autism (Stoner et al., 2014).

Disclosures: C. McSweeney: None. **D. Reynolds:** None. **A. Sebastian:** None. **J. Vitale:** None. **Y. Zhou:** None. **F. Dong:** None. **D. Deng:** None. **L. Liu:** None. **X. Jiang:** None. **J. Jiang:** None. **Y. Wang:** None. **I. Albert:** None. **Y. Mao:** None.

Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Title: Widespread neurodevelopmental malformations of the cerebellar vermis in genetically engineered mice

Authors: *J. A. CUOCO[1], A. ESPOSITO[1], S. SETH[1], S. O'MALLEY[1], Y. TANG[1], P. T. SMITH[2], R. L. RAMOS[1];
[1]Biomed. Sci., New York Inst. of Technol. Col. of Osteop, Old Westbury, NY; [2]Math & Natural Sci., SUNY Suffolk County Community Col., Brentwood, NY

Abstract: C57BL/6 mice are among the most widely used mouse strains in neuroscience research. This strain is often used as a "control strain" in a wide range of behavioral, anatomical, and physiological studies and is often the background strain used in the production of knock-out and transgenic mouse lines. In addition, a significant amount of research funding from the NIH is supporting projects using C57BL/6 mice. Work in our laboratory has recently described the presence of spontaneous malformations of the cerebellar vermis in C57BL/6 mice (Mangaru et al. 2013; Van Dine et al. 2013). Malformations consist of heterotopia containing a diverse array of neuronal (Purkinje cells, granule cells, Golgi cells, etc) and glial (astrocytes, Bergmann glia, oligodendrocytes) cell-types located in the molecular layer between lobules VIII and IX. Heterotopia are associated with disruption of Bergmann glial radial fibers as well as breeches of the pia along the secondary fissure and are indicative of neuronal migration defect. In a previous study we tested the prediction that mutant and transgenic mouse models on a C57BL/6 background would also exhibit these same cerebellar malformations (Ramos et al. 2013). Consistent with our hypothesis, we found that spontaneous mutant mice and several genetically-engineered mouse lines did indeed exhibit molecular layer heterotopia. In the present study, we expand the list of genetically-engineered mice, developed on a C57BL/6 background, that display cerebellar heterotopia including numerous Cre-driver lines, reporter lines, and GCAMP/Channelrhodopsin transgenic lines. These data have implications for investigators wishing to use these mice in neuroscience research.

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Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: MOST Grant 103-2321-B-010-008

Title: Cdk12 regulates neurogenesis and late-born neuronal migration in the developing mouse cerebral cortex

Authors: *M.-J. FANN, H.-R. CHEN;
Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: The DNA damage response (DDR) pathways are critical to ensure that replication stress and other types of DNA lesions do not perturb production of neural cells during development. Neural progenitors are particularly sensitive to DNA damage and their relative susceptibility may vary depending on the stage of development. Defective DDR is often associated with neurodevelopmental diseases. Cdk12 maintains genomic stability via regulation of expression of DDR genes. To define its role during neural development *in vivo*, we conditionally targeted Cdk12 using a Nestin-Cre mouse line. Nestin-Cdk12-cKO mice showed microcephaly and rarely survived beyond postnatal day 0 (P0). Results from cresyl violet-stained brain sections of Nestin-Cdk12-cKO at P0 showed reduced thickness of cortical plate layers in neocortex, and aberrant anterior commissure and corpus callosum. Nestin-Cdk12-cKO mice exhibited fewer neurons throughout the neocortex due to apoptosis of newborn progenitors. Furthermore, birthdating experiments with EdU showed that Cdk12 involved not only in survival of neural progenitor cells, but also in neuronal migration of late-born neurons in the developing neocortex. Although 6-cortical layer organization was preserved, misaligning of layers II-IV neurons marked with CUX1 expression was observed. An increase of ectopically localized CUX1+ cells in deeper layers was detected in Nestin-Cdk12-cKO neocortex. DiI tracing also showed a loss of the corpus callosum that is composed of axons of callosal projection neurons located in layer II/III. To further investigate effects of Cdk12 on neuronal migration without its confounding effect on neurogenesis, we used the Cdk12fx/fx mice that were electroporated *in utero* with Cre-expression plasmid between embryonic day 14 to 16. Migrating neurons are stagnant in the middle of the neocortex upon deletion of Cdk12. In short, Cdk12 function is crucial for two aspects of neuronal development, including neurogenesis and late-born neuronal migration. How deleting Cdk12 generates these effects is currently under investigation.

Disclosures: **M. Fann:** None. **H. Chen:** None.

Poster

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NRF-2013R1A1A2074251

Title: Regulation of the actin cytoskeleton by the Ndel1-Tara complex is critical for cell migration and invasion

Authors: J.-H. HONG[1], Y.-D. KWAK[1], Y. WOO[1], S. KIM[1], C. PARK[1], S.-A. LEE[1], K. SANADA[2], M. NGUYEN[3], *S. PARK[1];
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Abstract: Nuclear distribution element-like 1 (Ndel1) plays pivotal roles in diverse biological processes and is implicated in the etiology of multiple neurodevelopmental disorders. Ndel1 exerts its functions by regulating microtubules or intermediate filaments; however, its functional link to the actin cytoskeleton is largely unknown. Here, we show that Ndel1 interacts with TRIO-associated repeat on actin (Tara), an actin-bundling protein, to regulate cell movement. *In vitro* cell migration assays revealed that Ndel1- or Tara-deficient cells were defective in their migration and invasion. Moreover, Tara alters its intracellular distribution and this redistribution of Ndel1 was abolished by deletion of the Ndel1-interacting domain of Tara, suggesting that the altered localization pattern of Ndel1 requires a physical interaction with Tara. These findings uncover an interesting role of the Ndel1-Tara complex in actin reorganization during cell migration and invasion.

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Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: JSPS KAKENHI Grant Number 26830012

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Title: Cell mechanics underlying nuclear translocation of migrating neurons

Authors: *H. UMESHIMA[1], Y. K. WU[2], K. NOMURA[3], S. YOSHIKAWA[3], S. SAKUMA[4], F. ARAI[4], M. KANEKO[3], M. KENGAKU[1,2];
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Abstract: Neuronal migration is a fundamental step during brain development, whose defects are consistently correlated with brain malformation and dysfunctions. An essential feature of neuronal migration is the nuclear translocation. When neurons migrate, they at first elongate a long and thick process, referred to as a leading process, toward their migrating direction and then the nucleus (and other organelles) translocates into the leading process, as if a soft ball moves in a thin tube. Mechanical forces generated by microtubule and/or actin motor proteins have been implicated in the nuclear translocation, yet the precise mechanism is still unclear; it remains controversial which of microtubule or actin motors generate the primary force driving the nuclear translocation and whether these motor proteins push or pull the nucleus. In order to elucidate the physical basis of nuclear migration, we carried out high-resolution time-lapse imaging of nuclear dynamics in migrating cerebellar granule neurons. Our time-lapse imaging studies demonstrate that the nucleus dynamically changes its morphology and sometimes rotates during neuronal migration. Considering the nucleus as a viscoelastic body in a fluid dynamic system, these dynamic behaviors should reflect the forces applied to the nucleus. Hence, we have established digital image analyses in order to quantify the deformation and rotation of the nucleus. In addition, we have also measured the traction force on the culture substrate exerted by migrating neurons, using a modified traction force microscopy technique. It has been revealed that the leading process frequently exerts bidirectional traction force, contrasting with the unidirectional force of the trailing process. Furthermore, the traction forces generated in the leading process often synchronize and counteract to the nuclear translocation, suggesting that the pulling force contributes to nuclear translocation. In this presentation, we would like to discuss the force driving nuclear translocation based on these quantitative image analyses.

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Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01NS078164

Title: Identifying molecular mechanisms behind local cortical microcircuit assembly

Authors: *S. LODATO[1,2], L. GOFF[2,3], A. C. ZHANG[1], A. GROFF[1], E. J. STRONGE[1], A. SHETTY[1], J. RINN[1], P. ARLOTTA[1,2];
[1]Stem Cell and Regenerative Biol., Harvard Univ., Cambridge, MA; [2]The Broad Inst. of MIT and Harvard, Cambridge, MA; [3]John Hopkins University, Sch. of Medicine,, Baltimore, MD 21205, USA, MD

Abstract: The activity and function of the mammalian cerebral cortex rely on the precise assembly of specialized neuronal circuits involving an extraordinary diversity of excitatory projection neurons and inhibitory interneurons. Although they have different developmental origins, these two classes of neurons ultimately co-reside in the cortex, where they assemble into balanced local microcircuitry. The developmental events governing the proper interaction between excitatory projection neurons and inhibitory interneurons are poorly understood, in particular the cellular and molecular events that direct interneurons to position precisely within specific cortical layers. We recently reported that different subtypes of projection neurons uniquely and differentially determine the laminar distribution of cortical interneurons. We found that in Fezf2$^{-/-}$ cortex, the specific absence of subcerebral projection neurons and their replacement by commissural projection neurons causes abnormal lamination of interneurons and altered GABAergic inhibition. In addition, experimentally-induced ectopic corticofugal or callosal neurons selectively recruit distinct subtypes of cortical interneurons, based on the identity of the projection neuron. These data demonstrate that, in the cerebral cortex, individual populations of projection neurons cell-extrinsically control the laminar fate of interneurons and the assembly of local inhibitory circuitry (Lodato et al., Neuron, 2011). Here, we investigated the molecular mechanisms that mediate the precise pairing between distinct subtypes of projection neurons and interneurons in the early stages of radial migration of cortical interneurons. To address this question, we FACS-purified and transcriptionally profiled pairs of projection neuron and interneuron subtypes which preferentially reside in the same cortical layers, over multiple developmental time points. Using custom bioinformatic analysis, we have identified candidates that are functionally relevant for the lamination of interneurons within the cortex, and therefore crucial for the assembly of specialized cortical circuits. These molecules have potential roles in the etiology of neurological disorders such as autism and schizophrenia.

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Poster

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Program#/Poster#: 661.24/A60

Topic: A.01. Neurogenesis and Gliogenesis

Title: Novel function of PIWIL1 in neuronal polarization and migration via regulation of microtubule-associated proteins

Authors: *P. ZHAO[1], M. YAO[1], S. CHANG[1], L. GOU[2], M. LIU[2], Z. QIU[1], X. YUAN[3]; [1]Inst. of Neuroscience, CAS, Shanghai, China; [2]Inst. of Biochem. and Cell Biology, CAS, Shanghai, China; [3]Hussman Inst. for Autism, Baltimore, MD

Abstract: Young neurons in the developing brain establish a polarized morphology for proper migration. The PIWI family of piRNA processing proteins are considered to be restrictively expressed in germline tissues and several types of cancer cells. They play important roles in spermatogenesis, stem cell maintenance, piRNA biogenesis, and transposon silencing. Interestingly a recent study showed that *de novo* mutations of PIWI family members are strongly associated with autism. Here, we report that PIWI-like 1 (PIWIL1), a PIWI family member known to be essential for the transition of round spermatid into elongated spermatid, plays a role in the polarization and radial migration of newborn neurons in the developing cerebral cortex. Knocking down PIWIL1 in newborn cortical neurons by *in utero* electroporation of specific siRNAs resulted in retardation of the transition of neurons from the multipolar stage to the bipolar stage followed by a defect in their radial migration to the proper destination. Domain analysis showed that both the RNA binding PAZ domain and the RNA processing PIWI domain in PIWIL1 were indispensable for its function in neuronal migration. Furthermore, we found that PIWIL1 unexpectedly regulates the expression of microtubule-associated proteins in cortical neurons. Our finding of PIWIL1's function in neuronal development implies conserved functions of molecules participating in morphogenesis of brain and germline tissue and provides a mechanism as to how mutations of PIWI may be associated with autism.

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Poster

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Support: R01NS073112

Shriners Hospitals for Children Research Grant (85410)

Title: Yes-associated protein (Yap) function in normal cerebellar development and medulloblastoma

Authors: *L. J. HUGHES;
Temple Univ., Philadelphia, PA

Abstract: The Hippo signaling pathway is known to be involved in the control of organ size by regulating proliferation and apoptosis. This well conserved pathway is activated by various signal inputs, including cell-cell contact, mechanotransduction, and G-protein coupled receptors, with the signals converging on the downstream effector protein Yap and its homologue Taz. Yap has also been implicated as a potential oncogene, as it is upregulated and transcriptionally active in several tumor types including medulloblastoma, a tumor that arises in the cerebellum. Furthermore, inhibiting Yap activity in various cancer models has been shown to revert tumorigenic properties. Although the role of Yap has been described in several organ systems, there is a paucity of information about its role in the central nervous system. Here we investigate the function of Yap/Taz in the cerebellum to determine its significance during both normal development and medulloblastoma. To date, we have identified the expression pattern of Yap from embryonic through adult stages in mice. Although Yap is highly expressed in granule neuron progenitors (GNPs) during the rapid postnatal expansion stage, it does not appear to play a major role in proliferation of these cells as conditionally knocking-out Yap/Taz in GNPs does not alter their proliferative capacity. However, Yap/Taz does play a significant role in cerebellar foliation: Yap has an important function in Bergmann glia (BG) cells for establishing normal cerebellar foliation patterns during development. Furthermore, Yap-deficient BGs exhibit migrational defects. To determine the role of Yap in medulloblastoma, we utilized mouse models with constitutively activated sonic hedgehog (SHH) signaling, a pathway known to be activated in about 25% of medulloblastomas, in various cerebellar cell types. Because GNPs proliferate in response to SHH during normal development, SHH-mediated medulloblastoma is believed to originate from GNPs with dysregulated SHH signaling. We found that knocking out Yap/Taz in SHH-mediated medulloblastoma does not rescue the phenotype, an unexpected finding amidst several reports of the significant function of Yap in several other cancer types. Our observations demonstrate Yap has a novel function in cerebellar glia that is required for the development of normal foliation and organization, but plays a minimal role in cerebellar tumorigenesis.

Disclosures: L.J. Hughes: None.

Poster

661. Cell Migration in Neurodevelopment

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JST A-STEP AS2621606Q

Title: ADAMTS-3 is the major protease that inactivates Reelin in brain

Authors: *H. OGINO, A. HISANAGA, Y. KONDO, H. TSUIJI, T. KOHNO, M. HATTORI; Biomed. Science, Grad. Sch. of Pharmaceut. Scienses, Nagoya City Univ., Nagoya, Japan

Abstract: Reelin is a secreted glycoprotein that is essential for normal brain development and function. Reelin exerts its function by inducing phosphorylation of an intracellular protein Dab1. Phosphorylated Dab1 is degraded promptly. In recent years, downregulation and/or hypoactivity of Reelin in adult brain has been suggested to be involved in the pathogenesis of neuropsychiatric diseases including Alzheimer's disease and schizophrenia. Reelin protein is specifically cleaved at three sites, called N-t, C-t and CTR sites. The N-t site cleavage virtually abolishes Reelin's ability to phosphorylate Dab1. Therefore, inhibition of N-t site cleavage can be a therapeutic strategy for neuropsychiatric diseases. We identified ADAMTS-3 (A Disintegrin And Metalloproteinase with Thrombospodin motifs-3) as the candidate protease in charge of N-t site cleavage by purification from the supernatant of cultured cortical neurons. Recombinant ADAMTS-3 has the ability to cleave N-t site of Reelin *in vitro*. In this study, we analyzed the knockout (KO) mice of ADAMTS-3 as well as conditional KO mice that lacks ADAMTS-3 only in the excitatory neurons of forebrain to verify its contribution to N-t site cleavage *in vivo* and to clarify the physiological significance of N-t site cleavage. It turned out that most of ADAMTS-3 KO mice die soon after birth. The amount of cleaved product of Reelin is significantly decreased in the embryonic ADAMTS-3 KO mice brain, indicating that ADAMTS-3 is the major enzyme that cleaves the N-t site in embryonic stages. Importantly, the amount of Dab1 is decreased in cerebral cortex of embryonic ADAMTS-3-KO mice, indicating that Reelin activity is enhanced. These results strongly suggest that ADAMTS-3 is the major protease that inactivates Reelin in embryonic mice brain. In order to analyze the contribution of ADAMTS-3 to N-t site cleavage in postnatal stages, we established conditional KO mice that lacks ADAMTS-3 only in the excitatory neurons of forebrain. This conditional KO mice lives to adulthood and does not show

obvious behavioral or growth abnormality. We are currently analyzing the brain of this ADAMTS-3 conditional KO mice. These studies will help us understand the physiological significance of N-t site cleavage *in vivo*, particularly in the pathogenesis of neuropsychiatric diseases.

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Poster

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Title: Prolonged hypoxia depletes SVZ neural stem/progenitor cell pools critical for cortical development in piglets

Authors: *P. D. MORTON[1,2], L. KOROTCOVA[1,2], B. LEWIS[3], V. KUMAR[1,2], F. SHAIKH[1,2], E. SHORT[1,2], J. ZHANG[4], S. MORI[4], J. A. FRANK[3], V. GALLO[2], R. A. JONAS[1,2], N. ISHIBASHI[1,2];
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Abstract: Congenital heart disease (CHD) is the leading birth defect worldwide, affecting nearly 1 in every 100 infants. A majority of patients suffering from severe/complex CHD display significant neurological deficits, primarily due to a restricted oxygen supply to the brain during fetal life or early infancy. The subventricular zone (SVZ) generates neural stem/progenitor cells (NSPCs) that replenish damaged neurons and glia in the brain throughout the human lifespan. The structural and cellular properties of the well-studied rodent SVZ are dissimilar from its human counterpart. The piglet brain is a powerful tool to study human brain development as it shares more metabolic and physiological similarities to humans than other large mammals. In

addition, the porcine brain displays a highly evolved, gyrencephalic neocortex absent in many other mammals. Here we determine the contribution of SVZ NSPCs to cortical development in a human-like, gyrencephalic porcine brain under normal physiological conditions and after prolonged hypoxia. We found that the porcine SVZ shares significant anatomical/structural similarities to the human SVZ; including nearly identical laminar organization with an astrocyte ribbon. The dorsolateral-SVZ contained the largest number of NSPCs and was the predominant proliferative region in early postnatal development. A majority of NSPCs in the SVZ region generated immature neurons that migrated to the frontal cortices and olfactory bulb, indicating that the SVZ contributes to cortical development. Neurospheres generated from this cell population also displayed multipotency and a tendency for neuronal differentiation. Following hypoxia, our MRI studies demonstrated a reduction in cortical volume and folding of the frontal cortex; a phenomenon commonly seen in CHD patients. A reduction in cell proliferation and neurogenesis was also seen in the SVZ. In addition, results from *in vivo* cell labeling demonstrated that hypoxia limits the contribution of SVZ-derived neurons to postnatal cortical development. Finally, a decrease in the number of immature neurons was displayed within the frontal cortices with no changes in apoptosis. Our data indicate that chronic hypoxia reduces the generation of neuronal producing NSPCs in the SVZ, which delays/impairs corticogenesis. Future studies aimed at determining the underlying molecular signaling mechanisms coordinating the endogenous response of regenerating SVZ NSPCs will be invaluable in developing novel therapeutic targets and approaches to improve the neurological deficits exhibited in CHD patients.

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Poster

661. Cell Migration in Neurodevelopment

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 661.28/A64

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS082262 to E.S.T.

Title: JNK signaling maintains the integrity of cortical interneuron migratory streams during corticogenesis

Authors: *A. K. MYERS, K. BAKER, J. P. SNOW, S. E. HICKLING, C. A. SMITH, E. S. TUCKER;
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Abstract: Cortical interneurons assemble into streams of tangentially migrating cells as they enter the nascent cerebral cortex. As development proceeds, interneurons depart from migratory streams on diagonal or radial trajectories in order to populate the cortical plate, where they eventually segregate into layers and make synaptic connections with other cortical neurons. Errors in this process can lead to inappropriate cortical wiring and cause cortical circuit disorders such as schizophrenia, autism, and epilepsy. Therefore, elucidating cellular and molecular mechanisms underlying the transition from tangential to radial modes of migration is critical for understanding normal and pathological brain development. Our laboratory previously demonstrated that the c-Jun N-terminal kinase (JNK) intracellular signaling pathway is important for the initial entry of interneurons into the cerebral cortex. Jnk1/2 conditional double knockout mice, where Jnk1 is conditionally removed from interneurons of Jnk2 knockouts, exhibited significant delays in cortical interneuron entry as well as disruption in migratory streams at embryonic day (E) 13.5. In the present study, we explore the involvement of JNK signaling in the maintenance of cortical interneuron migratory streams between E14.5 and E15.5 when interneuron migratory streams are well formed. Time-lapse imaging of organotypic slice cultures treated with a pan pharmacological inhibitor of JNK signaling, SP600125, showed dramatic evacuation of cortical interneurons from the subventricular zone stream, while interneurons in the marginal zone stream slowed and extended leading processes into the cortical plate. Additionally, analyses of SP600125-treated slice cultures showed dose-dependent dispersion of interneurons from migratory streams and a tangential to radial re-orientation of leading processes. Washout experiments revealed that stream dispersion is partially reversible upon recovery of JNK signaling, indicating JNK-inhibition disrupts guided migration of cortical interneurons. Finally, Jnk1/2 conditional double knockout mice were evaluated to determine if interneuron-specific JNK-deficiency leads to migratory stream dispersion *in vivo* and in *ex vivo* slice cultures. Indeed, preliminary data indicate that long-lasting alterations in the radial distribution of cortical interneurons are evident in cortices of JNK-deficient mice. Completion of this work will further clarify the developmental mechanisms underlying appropriate migration and lamination of cortical interneurons and hopefully provide insight into the origins of detrimental disorders of cortical connectivity.

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

Poster

662. Patterning and Cell Death

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 662.01/A65

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF GRFP

Title: Deciphering astrocyte spatial patterning cues

Authors: *V. M. PUNAL, F. BRECHA, J. KAY;
Duke Univ., Durham, NC

Abstract: A growing body of evidence demonstrates that astrocytes are critical players in neural circuit development, refinement, and function. To ensure all neurons have access to astrocytes, these glial cells become regularly distributed throughout neural tissue during development. The mechanisms that instruct the patterning of astrocytes into a uniformly-spaced network are not known. The objective of the current study is to elucidate the key cell biological events underlying the development of astrocyte patterning. In most regions of the nervous system, astrocytes are born in a particular location and migrate to fill adjacent tissue. Next they must adjust their local cell density, either by dividing, dying, or moving short distances, in order to produce the mature, uniform distribution. To assess the relative contributions of these cellular behaviors in astroctye patterning, we chose to focus on astrocytes of the retinal nerve fiber layer. The retina is an excellent model because in this tissue astrocytes arise from a point source (the optic nerve), exist in a monolayer, and can be easily manipulated *in vivo* or imaged *ex vivo*. Astrocytes are not the only retinal cells showing non-random distribution - each neuronal cell type is also distributed evenly over the retinal surface in a pattern known as a mosaic. Past work on neuronal mosaics provides us with quantitative tools to assess astrocyte spatial patterning. To begin our studies we used these tools to confirm that mouse astrocytes form a mosaic. We found that astrocytes are indeed non-randomly distributed in mature retina. Next we quantified the time course of astrocyte mosaic formation. We found that astrocytes pass through several developmental stages prior to arriving at a mature mosaic. These stages include periods of astrocyte chain migration (P1-P3), followed by the reorganization of astrocyte cell body position into honeycomb-like clusters (~P5), and finally an epoch of mosaic refinement (P5-10) that culminates in astrocyte somata regularity (P17). Additionally, during the course of mosaic development, there is a marked reduction in astrocyte density. These results indicate that both long- and short-distance migration, in addition to cell death, likely play roles in producing the astrocyte mosaic. Taken together, this work sets the stage for future experiments aimed at detailing the cellular and molecular mechanisms that astrocytes use to colonize neural tissues and to ensure their uniform distribution throughout those tissues.

Disclosures: **V.M. Punal:** None. **F. Brecha:** None. **J. Kay:** None.

Poster

662. Patterning and Cell Death

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 662.02/A66

Topic: A.01. Neurogenesis and Gliogenesis

Title: Effect of depleting microglia on developmental cell death in the mouse brain

Authors: *J. A. STRAHAN, N. G. FORGER;
Georgia State Univ., Atlanta, GA

Abstract: During the perinatal period, apoptotic cell death eliminates roughly fifty percent of the neurons originally generated in the mouse brain. We previously reported that for most forebrain regions, cell death peaks just after birth, then wanes by the end of the first week (Ahern et al., 2013). However, several forebrain regions have delayed peaks of cell death (P5-P7) and there are large (>10-fold) regional differences in the magnitude of cell death. It is not known what accounts for these differences in timing and magnitude of neuronal cell death. Microglia have recently been reported to play an active role in controlling neuronal cell death, although findings have been contradictory. While microglia apparently increase developmental neuronal death in the hippocampus and cerebellum (Marin-Teva et al., 2004; Wakselman et al. 2008), they are neuroprotective in primary sensory cortex (Ueno et al., 2013). We find that the density of microglia correlates with both the timing and magnitude of cell death in the mouse brain at P1 and P5. This is consistent with the interpretation that microglia cause cell death, but alternatively could simply reflect their role in phagocytosing dead neurons. To directly address whether microglia are required for neuronal cell death, we used clodronate liposomes (CL) to deplete microglia in the neonatal mouse brain. CL are lipid vesicles filled with clodronate. They are exclusively taken up by macrophages (microglia), and when accumulated, are cytotoxic. CL have been used extensively for *in vitro* and peripheral applications, but sparingly *in vivo* in the CNS. We injected a 1:1 dilution of a 5 mg/ml solution of CL, or control saline-filled liposomes, into the cerebral ventricles of mouse pups on P1. Brains were collected 24h later and alternate sections processed for immunohistochemical detection of Iba-1 (a microglial marker) or activated caspase-3 (AC3, a marker of cell death). CL markedly reduced the number of Iba1-stained cells throughout many regions of the brain. Based on thresholding of Iba1 immunoreactivity, we see a ~90% reduction in the septum and hippocampus, and a ~70% reduction in the hypothalamus. In adjacent sections stained for AC3, we did not see significant differences in the number of dying cells (counted stereologically) in the septum, hypothalamus or hippocampus. This suggests that microglia are *not* required for normal cell death, at least within

the time frame tested. We are currently testing shorter and longer time points after CL treatment, and performing AC3 counts on additional brain regions.

Disclosures: J.A. Strahan: None. **N.G. Forger:** None.

Poster

662. Patterning and Cell Death

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Grants-in-Aid for Scientific Research (B) (26293248), (C) (25461560) from the Japan Society for the Promotion of Science (JSPS)

The Mother and Child Health Foundation

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Title: Valproic acid exposure *in utero* causes neocortical dysgenesis: altered proliferation/differentiation pattern of neural progenitor cells

Authors: *K. FUJIMURA[1], T. MITSUHASHI[1], S. SHIBATA[2], S. SHIMOZATO[1], T. TAKAHASHI[1];
[1]Dept. of Pediatrics, [2]Dept. of Physiol., Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Valproic acid (VPA), one of the first-line therapeutic agents for a wide spectrum of epileptic syndromes, inhibits histone deacetylases (HDACs) and hence modifies expression profiles of a variety of genes including cell-cycle related ones. An array of evidence strongly suggests that VPA exposure *in utero* increases the risk of congenital malformations, autism spectrum disorders, and lowers intelligence quotient scores in human offspring. Taken together, VPA exposure *in utero* may impair the cell cycle kinetics of neural progenitor cells (NPCs) through its HDAC-inhibitory activity leading to cerebral neocortical dysgenesis. We administered VPA to pregnant CD-1 mice through drinking water throughout the course of pregnancy. The following sets of experiments were conducted in the primary somatosensory area

of the neocortices of the postnatal day (P) 21 mice (experiments a and b) or in the dorsomedial cerebral walls of the embryos (experiments b and c): a) the thickness of neocortices and the number of GABAergic and non-GABAergic neurons, b) the number of neurons produced on the embryonic day (E) 16 and their final distribution in the neocortices, c) probability of cell cycle exit (quiescent or Q fraction) of the NPCs of the ventricular zone during the course of neuronogenesis. On P21, the thickness of the superficial neocortical layers (i.e., layers II-IV) was found to be increased in the VPA-exposed mice compared to that in the controls: this was exclusively attributable to an increased number of non-GABAergic neurons in those layers. The number of GABAergic neurons was indifferent between the two groups. Whereas neurons produced on E16 in the VPA-exposed mice were distributed in the layer II as in the controls, the number of those neurons was significantly increased. The Q fraction of the VPA-exposed NPCs was decreased by 10-40% during the early-middle phases of neuronogenesis compared to that of the controls. We infer that the fundamental effects of VPA exposure *in utero* are to decrease the Q fraction and to increase the number of NPCs during the early-middle phases of neuronogenesis. As a result, the number of non-GABAergic neurons, that are produced from the NPCs during the terminal phase of neuronogenesis (i.e., on E16 and later) and destined for the superficial neocortical layers, will be increased. It is of note that VPA exposure *in utero* may impair cell cycle regulation of the NPCs, possibly through its HDAC-inhibitory activity and leads to the neocortical dysgenesis.

Disclosures: K. Fujimura: None. **T. Mitsuhashi:** None. **S. Shibata:** None. **S. Shimozato:** None. **T. Takahashi:** None.

Poster

662. Patterning and Cell Death

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Topic: A.01. Neurogenesis and Gliogenesis

Support: DC008856

Neuroscience Graduate Program Training Grant

Title: The transcription regulator Lmo3 is required for correct cell fate specification in the globus pallidus

Authors: *S. BISWAS, L. GAN;
Univ. of Rochester, Rochester, NY

Abstract: The external globus pallidus is an important nucleus of the basal ganglia; crucial for their function in health and disease. In Parkinson's disease, although the principal target of decreased midbrain dopaminergic neurons is the striatum, the most prominent pathophysiology in late-stage animal models of this disease and in human patients occurs in the GP, along with the STN (more correlated, synchronous patterns of neuronal firing as compared to healthy controls). Central to the function of the GP is its neuronal diversity. Recent studies have showed that while the functionally relevant neurons of the GP are all GABAergic, there are at least two non overlapping subtypes, based on their neurochemistry, anatomy and electrophysiological properties. "Prototypic" neurons can be identified by the expression of the transcription factors Nkx2.1 and Lhx6, and most of these neurons express the calcium binding protein parvalbumin (PV). Protypic neurons project to downstream basal ganglia nuclei like the subthalamic nucleus (STN). On the other hand, "arkypallidal" neurons can primarily be identified by their expression of the transcription factor FoxP2, and their axons arborize extensively across the striatum. Interestingly, the prototypic neurons, on an average, have higher firing rates while the arkypallidal neurons have lower firing rates. Lineage tracing has shown that the majority of PV+ neurons and about half of the non-PV+ neurons of the globus pallidus originate from the medial ganglionic eminence, while half of the non-PV+ neurons originate from the lateral ganglionic eminence. However, inspite of some inroads being made into the developmental origin of GP neurons, specific molecular factors that control cell fate specification and thus contribute to the neuronal diversity of this structure are so far, unknown. Our work shows that loss of the transcription regulator Lmo3, results in a dramatic reduction of PV+ neurons in the mouse GP. I am currently investigating changes in vesicular GABA transporter (vGAT) and gephyrin expression in the STN, a major downstream target of the PV-expressing globus pallidus neurons. As the GP forms the predominant inhibitory input of the STN, a decrease in the number of GP projections to the STN may have a significant impact on basal ganglia physiology and function.

Disclosures: **S. Biswas:** None. **L. Gan:** None.

Poster

662. Patterning and Cell Death

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NINDS grant R01 NS057536

Cerebral Palsy International Research Foundation Award

Title: Characterizing the role of adhesion G protein-coupled receptor GPR56 in cortical lamination

Authors: *M. OKAMOTO, R. LUO, X. PIAO;
Newborn Med., Boston Children's Hospital, Harvard Med. Sch., Boston, MA

Abstract: Defects in neural migration and cortical lamination during development are among the leading causes of intellectual disabilities and epilepsy in humans. How post mitotic neurons reach and stop at their destined laminar position during cortical development remains one of the most fundamental questions in developmental neuroscience. There are many kinds of interactions between cortical cells (neurons and progenitor cells) and their surrounding environment to regulate cortical lamination. Previous studies have shown that mutations in adhesion G protein-coupled receptor 56 (GPR56) cause a devastating human brain malformation called bilateral frontoparietal polymictogria (BFPP) and deletion of GPR56 and its ligand Collagen III lead to the overmigration of neurons through a regionally breached pial basement membrane (BM) in mice, suggesting that the interaction between GPR56-expressing cortical cells and BM regulates the pial BM integrity and cortical lamination during cortical development. In this study, we investigated how interactions between cortical cells and microenvironment coordinate the cell dynamics underlying the cortical histogenesis via analysis of GPR56 signaling. We first detected GPR56 expression in earliest born preplate neurons at embryonic day (E) 10. Furthermore, we discovered that the overmigration, which is accompanied by disruption of the BM, is induced by the deletion of GPR56 between E9 and E12 with a gradually reduced severity of the defect along the development through the analysis of tamoxifen-inducible GPR56 (fl/fl); Nestin-cre mice. These results suggest that GPR56 might play a role in neuronal migration, cortical lamination and the maintenance of the pial BM in a stage dependent manner.

Disclosures: **M. Okamoto:** None. **R. Luo:** None. **X. Piao:** None.

Poster

662. Patterning and Cell Death

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NSERC

NeuroDevNet

CIHR

CMMT/CFRI start-up funds

Title: Wntless is required for compartmentation and lamination in cerebellar development

Authors: *J. T. YEUNG[1,2], D. GOLDOWITZ[3,2];
[1]CMMT, Vancouver, BC, Canada; [2]Med. Genet., Univ. of British Columbia, Vancouver, BC, Canada; [3]Ctr. for Mol. Med. and Therapeut., Child and Family Res. Inst., Vancouver, BC, Canada

Abstract: The wnt signaling pathway involves a diversity of developmental processes in neural development. Previously, we demonstrated that Wntless (Wls), a transmembrane protein essential for the secretion of Wnt molecules, is expressed predominately in the cerebellar rhombic lip (RL) and isthmic organizer (ISO) during cerebellar development. Normally the expression of Wls is restricted to the interior face of the RL (iRL) by Pax6 expression, and we found that the Wls domain expands in the absence of Pax6. Together with the expression of Math1, Pax6, Lmx1a and Tbr2, Wls demarcates the RL into four molecularly distinct compartments. The fate of the cells from the Wls-positive compartment is unknown. The study of Wls in cerebellar development, however, is hindered by the early embryonic lethality of the conventional Wls-knockout. In this work, we employ the Cre-lox system to conditionally knockout *Wls* to circumvent the early requirement of Wls. By using Nestin-Cre, *Wls* is ablated conditionally from the cells of the RL at mid-gestation. With this *Wls*-cKO approach, Wls expression is deleted from the RL cells beginning at E12.5, while expression in the ISO is intact. The *Wls*-cKO mutant survives the embryonic period but dies within 6 hours after birth. Thus, this *Wls*-cKO allows for the study of Wls function in the RL and early cerebellar development. Examination of the *Wls*-cKO cerebellum at P0 reveals a significant reduction in the size of vermis, while the hemispheres are less affected. Across the whole cerebellum, the *Wls*-cKO lacks the formation of primary fissures and foliation is largely disrupted. The external granule layer (EGL) of the *Wls*-cKO is highly disorganized: at the medial region, gaps are observed between patches of granule cells, on the other hand, ectopic granule cells are observed in clusters that reside in the cerebellar core of the hemispheres. At the gaps in the EGL, we detected cells that are positive for Pax2, a marker of interneurons, or calbindin^{+}, a marker for Purkinje cells. Interneurons and Purkinje cells migrate through the gaps in the EGL and ectopically reach the surface of the cerebellum. The present work is the first to describe the cerebellar phenotype in the absence of Wls. Our findings indicate that Wls play a role in directing the proper placement of cerebellar cell types during development.

Disclosures: J.T. Yeung: None. **D. Goldowitz:** None.

Poster

662. Patterning and Cell Death

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Title: Differential dependency of hindbrain serotonergic neuron development on transforming growth factor betas

Authors: *E. CHLEILAT, E. ROUSSA;
Mol. Embryology, Univ. of Freiburg, Freiburg, Germany

Abstract: Hindbrain serotonergic (5-hydroxytryptamine, 5-HT) neurons contribute to the regulation of a wide range of behaviors and their dysfunction may lead to neurological disorders. The 5-HT neurons of the hindbrain exist in two groups: rostral and caudal. These two groups can be further divided into various subpopulations of raphe nuclei later in development. Interestingly, 5-HT subpopulations are selectively affected in clinical disorders. A better understanding of the development of 5-HT neurons might contribute to the advancement of selective therapies for neurological disorders. The cytokines transforming growth factor betas (TGF-βs) may influence 5-HT neuron development. The double knockout mice for TGF-β 2/3 display a phenotype in midbrain dopaminergic neurons, a neuronal subpopulation that shares common developmental signals with hindbrain 5-HT neurons. The aim of the present study was to investigate the role of TGF-βs in the specification, development and survival of hindbrain 5-HT neurons. Using Cre-lox technology, we generated 3 conditional knockout mice lines with the deletion of TGF-β receptor 2 (TGFBR2) and the ligand TGF-β2 (TGF-β2) in cell-type specific hindbrain regions. In $TGF\text{-}\beta2^{flox/flox}::Krox20^{Cre/+}$ and $TGFBR2^{flox/flox}::Krox20^{Cre/+}$, TGF-β2 and TGFBR2 were knocked out in rhombomeres 3 and 5, respectively. In the $TGFBR2^{flox/flox}::En1^{Cre/+}$, TGFBR2 was knocked out in rhombomere 1. From these animals, 5-HT immunopositive neurons were counted in the hindbrain groups and subpopulations. The results show that in the $TGFBR2^{flox/flox}::Krox20^{Cre/+}$ mice, absence of TGFBR2 causes no statistically significant difference in the number of 5-HT neurons in the rostral and caudal groups at embryonic day (E) 14 compared to control. Similarly, at E18, where the 5-HT neurons are further developed, again no statistical difference was seen in the number of 5-HT neurons in the various raphe nuclei subpopulations. At E14 in the $TGFBR2^{flox/flox}::En1^{Cre/+}$ mice, no statistical difference in the 5-HT groups was observed. However, a statistical decrease in the number of 5-HT neurons was seen at E18 in the subpopulations compared to control. The most robust phenotype seen was in the $TGF\text{-}\beta2^{flox/flox}::Krox20^{Cre/+}$ mice which reveal statistically significant decrease in the number of 5-HT neurons in the caudal group in comparison to control at E14 and in every subpopulation except the rostral dorsal raphe nucleus at E18. The results propose a differential TGF-β2 (and TGFβ

signaling) dependency for proper 5-HT neuron development. This preliminary phenotypic characterization will be completed by investigating the molecular mechanism.

Disclosures: **E. Chleilat:** None. **E. Roussa:** None.

Poster

662. Patterning and Cell Death

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NINDS R01NS070159

Title: Understanding the role of GABAergic signaling in larval zebrafish development

Authors: ***A. J. VANLEUVEN**[1], L. BEEBE[2], R. E. BALL[1], M. O'CONNOR[3], T. DORE[3], J. D. LAUDERDALE[1];
[1]Cell. Biol., [2]Genet., Univ. of Georgia, Athens, GA; [3]New York Univ., Abu Dhabi, United Arab Emirates

Abstract: γ-Aminobutyric Acid (GABA) is best known as the primary inhibitory neurotransmitter in the central nervous system of all vertebrates, but intriguing evidence has implicated GABA as a key signaling molecule in craniofacial development. Previous studies in mice show that *Gad1* mutants have severe cleft palates and do not live past birth, while *Gad2* mutants appear normal and viable, but exhibit stress-induced seizures. Due to neonatal lethality, the mechanism of *Gad1* activity in craniofacial development cannot be easily investigated in a mammalian model, but zebrafish provide an optimal system for such studies. Our work has focused on elucidating the function of the *gad1* and *gad2* genes in larval zebrafish through the use of morpholinos, novel photocaged morpholinos and CRISPR/Cas genome editing to alter GAD gene activity. In zebrafish, as in other vertebrates, there are two GAD genes, *gad1* and *gad2* that encode two isoforms of the GAD enzyme. Through the use of morpholinos, our lab has shown that *gad1* but not *gad2* morphants exhibit abnormal development of cranial neural crest cells. This phenotype can be bypassed using novel photocaged *gad1* morpholinos, when uncaging the morpholino at 1dpf. In these animals, craniofacial development appears largely normal, but electrophysiological recordings show increased and abnormal brain activity when compared to wild-type larvae. This evidence indicates that *gad1* is playing a role in early craniofacial development, which is independent of its role in GABA synthesis and

neurotransmission. We are currently validating these morpholino results with the several novel *gad1* and *gad2* alleles generated by the CRISPR/Cas method for targeted genome editing.

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Poster

662. Patterning and Cell Death

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Nancy Lurie Marks Family Foundation

Title: Developmental control of cortical GABAergic interneuron number via Pten signaling

Authors: ***J. SEJOURNE**, D. T. PAGE;
Neurosci. department, The Scripps Res. Inst. Florida, Jupiter, FL

Abstract: GABAergic interneurons are crucial for establishing the functional balance and computational architecture of cortical circuits. Dysfunction of interneurons is linked with a variety of neuropsychiatric disorders, including autism and schizophrenia. Interneurons are generated in the ventral telencephalon and migrate to the cerebral cortex, where they undergo an intrinsic program of cell death during development to arrive at a mature population size. Recent findings indicate that interneuron cell death in the developing cortex is regulated by a cell- or population-autonomous mechanism, which has not yet been identified. Given the role of Pten phosphatase in regulating cell death, we hypothesize that its downstream signaling, particularly through Akt-FoxO, may play a role in this process within interneurons. To test this possibility, we generated mice carrying conditional heterozygous mutations in Pten restricted to GABAergic cell types (Gad2-Cre+; Pten[loxp/+]). In wild-type mice, we found that the number of GABAergic cells in the cortex decreased by approximately 25% between postnatal day 4 (P4) and P14, consistent with previous reports. In contrast, cortical GABAergic cell number in Gad2-Cre+; Pten[loxp/+] mice decreased by only 4%. Both wild-type and mutant animals had an equivalent number of cortical GABAergic cells at P4, but there was a net increase in mutants at P14 that persisted into adulthood, indicating that conditional mutation of Pten in GABAergic interneurons influences the postnatal survival of these neurons in a cell autonomous manner. Corresponding with this cellular phenotype, we observed deficits in social approach and open field behavior in Gad2-Cre+; Pten[loxp/+] animals. We are currently investigating alterations in

pro-apoptotic Akt-FoxO signaling downstream of Pten as a mechanistic explanation for this effect. Our results provide insight into the mechanism that controls the population size of GABAergic neurons during development and the importance of keeping a balanced ratio between excitation and inhibition in the brain for cognitive functions.

Disclosures: J. Sejourne: None. **D.T. Page:** None.

Poster

662. Patterning and Cell Death

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Support: NIH R21NS085679

The Biological Sciences Collegiate Division Endowments at the University of Chicago

Title: Signaling centers that pattern developing cerebral cortex are conserved between mice and ferrets

Authors: *W. D. JONES, S. M. GUADIANA, E. A. GROVE;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: The mammalian cerebral cortex, including the neocortex and hippocampus, is responsible for higher cognitive functions such as decision-making, perception, and memory. Cortical regions that control these functions are specified in the developing embryo, when secreted factors from signaling centers in the brain direct cells in different regions of the developing cortex to adopt distinct fates. This model of cortical development, however, has been worked out in the lissencephalic mouse, and its relevance to cortical development in gyrencephalic species, including humans, is unknown. Differences between lissencephalic and gyrencephalic cortices suggest that patterning signals may contend with different spatial considerations in the two types of cortex. To begin to assess the relevance of area patterning mechanisms in the mouse to species with a gyrencephalic cortex, we used a gyrencephalic model, the ferret *Mustela putorius furo*. At various time-points of cortical development in the ferret, we characterized, with *in situ* hybridization, the expression patterns of several genes implicated in cortical patterning. These genes encode Fibroblast Growth Factors, Bone Morphogenetic Proteins, and Wnt proteins, along with Emx and Nr2f1 transcription factors. Comparison of the expression patterns in mice and ferrets at equivalent developmental stages

reveals strong similarities, providing evidence that highly similar signaling mechanisms could govern cortical area patterning in lissencephalic and gyrencephalic species. Furthermore, the ferret is more distantly related to rodents than are primates, making ferrets a good out-group for testing conservation of area patterning mechanisms. In particular, similar mechanisms shared by ferrets and rodents are likely to be conserved in humans and non-human primates.

Disclosures: W.D. Jones: None. **S.M. Guadiana:** None. **E.A. Grove:** None.

Poster

662. Patterning and Cell Death

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 662.11/A75

Topic: A.01. Neurogenesis and Gliogenesis

Support: National Basic Research Program of China (2013CB835100)

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National Natural Science Foundation of China (81222013)

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Title: The role of PDK1 in the development of mice dentate gyrus

Authors: X. HAN[1], M. XU[2], B. LIU[1], T. TIAN[2], C. ZHAO[1], *J. GAO[3];
[1]Southeast Univ., Nanjing, China; [2]Nanjing Med. Univ., Nanjing, China; [3]Nanjing Med. Univ., Jiangsu, China

Abstract: Hippocampus is a crucial cortical region and administers learning and memory. Dentate gyrus is the first gateway receiving afferent input from the Entorhinal Cortex. The signal comes out from dentate gyrus to the CA zones then comes back to dentate gyrus which constitutes the classic circuit to form memories. Its substructure SGZ (subgranular zone) harbors the Tbr2 positive neuronal progenitors which can generate neurons throughout adulthood when occurs to enriched environment, stress and so on. Moreover these new born neurons can integrate into the existed neural circuit then perform the normal function. 3-phosphoinositide-dependent protein kinase-1 (PDK1) is a regulator of AGC kinases compromised with PKA, AKT (PKB) and S6K et al. PDK1 phosphorylates the highly conserved sequences on their T-loops by which to activate these protein kinases, and regulates cell apoptosis,survival,glucose uptake and storage and so on in mammals. There is no much research focused on the function of PDK1 in nervous

system. Just a few papers reported that ablation of PDK1 in nestin+ cells would result in the microcephaly. To learn more about the function of PDK1 in brain we used the Cre-Loxp approach to knock out PDK1 in Emx1 positive cells conditionally which are expressed in the dorsal telencephalon. We find the mutant mice have weaker capacity of learning and memory associated with hippocampus. Through morphology approaches the mutant postnatal hippocampus is found to have a smaller size than control. DG is the most severely impacted substructure with abnormal appearance more than decreased cell numbers. We use immunohistochemical method to detect the function of PDK1 before birth and find the ablation of PDK1 impacts the development of DG via reducing proliferation,increasing differentiation and hypogenetic radial glial scaffold. PDK1 could phosphorylate the Thr308 site of AKT whose Ser473 is phosphorylated by mTOR2. AKT is proved to be related with the survival and proliferation of neurons and mTORC2 is found to participate in the formation of dendritic of neurons. It is significant that if PDK1 modulate the development of DG via controlling these two signals.

Disclosures: **X. Han:** None. **M. Xu:** None. **B. Liu:** None. **T. Tian:** None. **C. Zhao:** None. **J. Gao:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 663.01/A76

Topic: A.02. Postnatal Neurogenesis

Support: Stockton University REU Grant

Stockton University Research and Professional Development Grant

Title: Activation of the mTOR pathway drives neurogenesis and alters neuronal fate in the olfactory bulb

Authors: ***N. W. HARTMAN**, A. GUBISTA, A. DECARLO, D. HELLTHALER;
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Abstract: Neural stem cells (NSCs) of the subventricular zone (SVZ) give rise to inhibitory interneurons in the olfactory bulb based on their location in the SVZ. Recent studies have shown that activation of the mammalian target of rapamycin (mTOR) pathway can drive NSCs to differentiate and alter dendritic morphology. Here, we targeted NSCs either in the dorsal or

ventral SVZ by postnatal electroporation. Stem cells in the dorsal SVZ were more likely to produce periglomerular neurons, whereas ventral SVZ cells primarily gave rise to granule cells. In addition, activation of mTOR resulted in increased dendritic complexity in both periglomerular cells. Interestingly, driving mTOR increased overall neurogenesis only in the dorsal SVZ. In contrast, increased mTOR activity bolstered the production of periglomerular cells from the ventral SVZ, but did not the dorsal SVZ. Isolated dorsal NSCs in culture display increased mTOR pathway activity in addition to increased dorsal gene expression compared to their ventral counterparts. These results suggest that mTOR can affect regional identity of NSCs, either driving neurogenesis or altering the cell fate of progenitors.

Disclosures: **N.W. Hartman:** None. **A. Gubista:** None. **A. DeCarlo:** None. **D. Hellthaler:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.02. Postnatal Neurogenesis

Support: FCT, Portugal, COMPETE and FEDER

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Title: Identification of neurogenic S-nitrosylation targets in neural stem cells

Authors: *I. M. ARAUJO[1], A. I. SANTOS[2], D. M. SILVA[2], A. S. LOURENÇO[2], A. IZQUIERDO-ÁLVAREZ[3], E. RAMOS[3], C. M. CARVALHO[4], A. MARTÍNEZ-RUIZ[3]; [1]Dept. of Biomed. Sci. and Med., Univ. of Algarve, NIF 505 387 27, Faro, Portugal; [2]Dept. of Biomed. Sci. and Med., Univ. of Algarve, Faro, Portugal; [3]Servicio de Inmunología, Hosp.

Universitario de la Princesa, Inst. de Investigación Sanitaria Princesa, Madrid, Spain; [4]Ctr. for Neurosci. and Cell Biol., Coimbra, Portugal

Abstract: Nitric oxide (NO) is a well-established regulator of neurogenesis. NO enhances proliferation of neural stem cells (NSC), and is essential for hippocampal injury-induced neurogenesis following an excitotoxic lesion. Although the main effects of NO on NSC proliferation are via activation of the ERK/MAPK and cGMP/sGC/PKG pathways, S-nitrosylation may have a substantial role in the activation and/or inhibition of several proteins involved in the neurogenic process. Protein S-nitrosylation is a post-translational modification that consists in the formation of a nitrosothiol group (R-SNO) in cysteine residues, which can promote formation of other oxidative modifications in those cysteine residues. It is one of the mechanisms underlying non-classical NO cell signaling that regulate many physiological processes, including neuronal plasticity. The aim of this work is to identify proteins modified by S-nitrosylation in conditions that promote cell proliferation in NSC derived from the subventricular zone, and that could take part in non-classical NO signaling. Treatment with S-nitroso-L-cysteine (CysSNO), a physiological permeable nitrosothiol, increased protein cysteine oxidation and S-nitrosylation in NSC, as assessed by a fluorescence switch assay. Separation by two-dimensional electrophoresis and analysis by mass spectrometry resulted in the identification of several proteins that were modified by treatment with CysSNO. From those, p21Ras, PEBP-1, PCNA, 14-3-3 proteins and hnRNP K, were the focus of further validation due to their relevance in the neurogenic context, including their involvement in the ERK/MAPK pathway. By using the biotin switch technique, we show a strong increase in oxidation and, specifically, in S-nitrosylation signal of p21Ras, PEBP-1, PCNA, 14-3-3 and hnRNP K in the presence of CysSNO. Overall, this work identifies several proteins as a target of S-nitrosylation in NSC and suggests new candidates for NO-induced regulation of neurogenesis.

Disclosures: **I.M. Araujo:** None. **A.I. Santos:** None. **D.M. Silva:** None. **A.S. Lourenço:** None. **A. Izquierdo-Álvarez:** None. **E. Ramos:** None. **C.M. Carvalho:** None. **A. Martínez-Ruiz:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 663.03/A78

Topic: A.02. Postnatal Neurogenesis

Support: NRF Grant 2012R1A2A2A01046132

Title: Zinc transporter 3 gene deletion alters adult hippocampal neurogenesis

Authors: *B. CHOI[1], I. KIM[1], B. LEE[1], J. KIM[1], A. KHO[1], S. LEE[2], M. SOHN[3], S. SUH[1]; [1]Dept. of Physiol., [2]Dept. of Neurol., Hallym University, Col. of Med., Chuncheon, Korea, Republic of; [3]Dept. of Nursing, Inha Univ., Incheon, Korea, Republic of

Abstract: The subgranular zone (SGZ) of the dentate gyrus (DG) is the most active neurogenic region and also contains highly concentrated synaptic zinc in the mossy fiber terminals. We therefore tested the hypothesis that vesicular zinc is an important mediator for neurogenesis in this system. In support of this hypothesis, our previous studies demonstrated that hippocampal zinc is an important factor for neurogenesis in the adult brain. Application of zinc chelator and zinc-deprived diet reduced progenitor cell proliferation and neurogenesis. In addition, evidence has accumulated from numerous studies indicating that zinc is an essential trace element required in cell division, proliferation, migration, and development and further suggests that zinc may have a critical role in neurogenesis and cognitive function. To determine whether synaptic zinc is important for modulating hippocampal neurogenesis, the present study used zinc transporter 3 (ZnT3) gene deletion mice. ZnT3 is a zinc transporter that is located in the synaptic vesicle membrane and regulates levels of ionic zinc within presynaptic terminals in the hippocampus. ZnT3 knockout (KO) mice show marked depletion of synaptic vesicular zinc. Here we report that endogenous ZnT3 regulates adult hippocampal neurogenesis. Neurogenesis was evaluated using BrdU, Ki67 and doublecortin (DCX) immunostaining. Genetic deletion of ZnT3 reduced the number of BrdU and Ki67 positive cells in the SGZ of the DG. Further, we found that ZnT3 deletion reduced the number of DCX positive cells. Our findings suggest a profound role of ZnT3 in the regulation of adult hippocampal neurogenesis under physiological conditions.

Disclosures: B. Choi: None. **I. Kim:** None. **B. Lee:** None. **J. Kim:** None. **A. Kho:** None. **S. Lee:** None. **M. Sohn:** None. **S. Suh:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

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Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant MH090258

Title: A maturational transition of distict Wnt signals enables a stage-specific regulation of dentate granule cell neurogenesis

Authors: *S. T. SCHAFER, J. HAN, M. PENA, F. H. GAGE;
LOG-G, Salk Inst. For Biol. Studies, La Jolla, CA

Abstract: The dentate gyrus (DG) of the hippocampus is one of the stem cell housing niches in the adult mammalian brain. Canonical Wingless-type (Wnt) signals provided by the microenvironment are one of the major niche factors that regulate the differentiation of adult neural stem cells (aNSCs) towards the neuronal lineage. Wnts are part of a complex and diverse set of signaling pathways with a wide range of possible interactions. It remains unknown whether different canonical and non-canonical Wnt signals act in a stage-specific manner to regulate distinctive steps of adult hippocampal neurogenesis. Using *in vitro* assays on adult hippocampal NSCs, we identified an attenuation of canonical Wnt/ß-Catenin signaling responsiveness in the course of neuronal differentiation, while non-canonical Wnt/Planar Cell Polarity (PCP) signaling events progressively increased. Retroviral knockdown strategies against ATP6AP2, a recently discovered core protein involved in both signaling pathways, revealed that its dual role is critical for granule cell fate and morphogenesis. We were able to confirm its dual role in neurogenic Wnt signaling *in vitro* for both canonical Wnt signaling in proliferating aNSCs and non-canonical Wnt signaling in differentiating neuroblasts. While LRP6 appeared to be critical for granule cell fate determination, *in vivo* knockdown of PCP core proteins FZD3 and CELSR1-3 revealed severe maturational defects without changing the identity of newborn granule cells. Furthermore, we found that CELSR1-3 control distinctive aspects of PCP-mediated granule cell morphogenesis with CELSR1 regulating the direction of dendrite initiation sites and CELSR2/3 controlling radial migration and dendritic patterning. In summary, the data presented here characterize a transition of Wnt signaling responsiveness from Wnt/ß-Catenin signaling to non-canonical Wnt/PCP signaling in the course of granule cell fate that was also present in a human pluripotent stem cell (hPSC)-based model of dentate granule neurogenesis. Thus, our work reveals temporally distinct roles for different Wnt signaling pathways that may be achieved through a cell-autonomous mechanism to integrate niche-derived Wnt signals in a stage-specific manner.

Disclosures: **S.T. Schafer:** None. **J. Han:** None. **M. Pena:** None. **F.H. Gage:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 663.05/A80

Topic: A.02. Postnatal Neurogenesis

Title: The interaction of neuronal Sema6D and microglial Plexin-A1 in developmental neuronal death in hippocampus

Authors: *T. ITO, K. YOSHIDA, T. NEGISHI, K. YUKAWA;
Meijo Univ., Nagoya / Aichi, Japan

Abstract: In the developing hippocampus, microglia actively promotes apoptotic neuronal death. The microglial DAP12 immunoreceptor and CD11b integrin cooperatively induce neuronal apoptosis by controlling the production of superoxide ions (Wakselman S et al, 2008). Our coimmunoprecipitation assay has disclosed the association of DAP12 with the Plexin-A1 semaphorin receptor and TREM2 in postnatal microglia. However, the role of Plexin-A1 in developmental neuronal death remains unknown. In the developing hippocampus, apoptotic neurons were in contact with microglia expressing DAP12, Plexin-A1 and TREM2. There was a significant decrease of apoptotic cells in the Plexin-A1-deficient hippocampus at postnatal day 1-3 (PND1-3) as compared with wild-type (WT). The production of superoxide ions was significantly decreased in Plexin-A1-deficient microglia reflecting the dependency on Plexin-A1 signaling. The expression level of Sema6D, one of Plexin-A1 ligands was significantly higher in hippocampi at PND1-3 compared with other developmental stages. Furthermore, activated caspase-3-positive neurons exhibited the increase of Sema6D expression in hippocampi at PND1-3. Addition of recombinant Sema6D significantly increased the production of reactive oxygen species (ROS) in cultured WT microglia, but not in Plexin-A1-deficient microglia. Sema6D induced a significant increase of neuronal apoptosis in cocultures of WT neurons and WT microglia as compared with the immunoglobulin G control, which was significantly inhibited by the free radical scavenger N-tert-butyl-phenylnitrone. In contrast, Sema6D did not induce a significant increase of neuronal apoptosis in cocultures of WT neurons and Plexin-A1-deficient microglia. The expression level of Sema6D also increased in cultured neurons in which the firing was lowered by tetrodotoxin (TTX). Microglial ROS production significantly increased in cocultures of WT neurons pretreated with TTX and WT microglia, but not in cocultures of WT neurons pretreated with TTX and Plexin-A1-deficient microglia. Cocultures of WT neurons pretreated with TTX and WT microglia displayed a significant increase of apoptotic neurons as compared with cocultures of WT neurons without TTX treatment and WT microglia. In contrast, there was no significant increase of neuronal apoptosis in cocultures of WT neurons pretreated with TTX and Plexin-A1-deficient microglia. Thus, Sema6D generated by dying neurons may function as a kill me signal to induce developmental neuronal apoptosis by activating microglial Plexin-A1-mediated signal which promotes ROS production.

Disclosures: **T. Ito:** None. **K. Yoshida:** None. **T. Negishi:** None. **K. Yukawa:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 663.06/A81

Topic: A.02. Postnatal Neurogenesis

Title: A role for lipocalin-2 in hippocampal neurogenesis modulation

Authors: *P. MORGADO[1,2], A. C. FERREIRA[1,2], S. D. MESQUITA[1,2], A. NOVAIS[1,2], S. NEVES[1,2], N. SOUSA[1,2], J. A. PALHA[1,2], J. C. SOUSA[1,2], F. MARQUES[1,2];
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Abstract: Stress is defined as a challenge to the homeostatic equilibrium of the organism and when long lasting it severely affects quality of life. In fact, stress maladaptation is considered to be an etiological factor in the emergence of mood disorders like 5depression and anxiety. Stress response elicits a cascade of hormonal and behavioral changes as an attempt to maintain homeostasis and at the level of the central nervous system (CNS), the hippocampus is considered to be a central target and executor of adaptative responses. Of interest, the hippocampus is unique in its structural and cellular plasticity, in the sense that, even in adulthood, stem cells reside and continuously give rise to new neurons. This adult neurogenesis is extremely dynamic and modulated by pharmacological, environmental and physiological stimuli and the newborn neurons are considered to highly contribute to the local network. Of interest, adult neurogenesis is well known to be regulated by stress and, conversely, to regulate stress responses. Of course there is no simple, linear relationship between stress hormones and adult neurogenesis. As an attempt to disclose putative factors in this crosstalk of stress modulation, in the present work we have assessed the modulation of hippocampal neurogenesis in an animal model with a targeted deletion of lipocalin-2 (LCN2). As part of the lipocalin protein family, LCN2 plays a critical role in the regulation of various phyisiological processess, such as inflammation and innate immune response. Several roles for cell proliferation and death have also been attributed to this protein, including during development, in hematopoiesis and even cancer, mainly recognized due to the capacity of LCN2 to traffic iron within cells. In addition, LCN2-null mice were described, at a physiological level, to present a sustained activation of the HPA axis translated into increased levels of corticosterone, accompanied by an anxious and depressive-like phenotypes. Also it was described to control anxiety and neuronal excitability upon stress. But specifically how is hippocampal neurogenesis being modulated upon LCN2 deletion and to which extent it contributes to animal's behavior and stress response is not quite known. Here we show that LCN2-null mice exhibit cell proliferation deficits, accompanied by impairments in adult neurogenesis progress and in contextual fear conditioning paradigms. The current observations will certainly contribute to the current interests on the role of hippocampal neurogenesis in the

etiology of mood, identifying LCN2 as a possible key regulator of executor of adaptative responses at the hippocampus.

Disclosures: **P. Morgado:** None. **A.C. Ferreira:** None. **S.D. Mesquita:** None. **A. Novais:** None. **S. Neves:** None. **N. Sousa:** None. **J.A. Palha:** None. **J.C. Sousa:** None. **F. Marques:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

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Topic: A.02. Postnatal Neurogenesis

Support: KRF 2640044

Title: Sp8 and Sp9 coordinately regulate olfactory bulb interneuron development

Authors: ***J. LI**, C. WANG, D. QI, Y. YOU, Z. YANG;
Fudan Univ., Shanghai, China

Abstract: The zinc finger transcription factor Sp8 is wildly expressed in the subventricular zone, rostral migratory stream and olfactory bulb (SVZ-RMS-OB system). Conditional inactivation of Sp8 in the embryonic ventral telencephalon reveals that Sp8 is required for the normal generation of some OB interneuron subtypes. Herein, we show that Sp9 is also broadly expressed in the SVZ-RMS-OB system. Sp9 is not expressed in neural stem cells and transit-amplifying progenitors (type C cells) in the postnatal SVZ; however, all neuroblasts express Sp9. In Sp9 mutant OB, parvalbumin-expressing interneurons are lost. Only one amino acid residue differs between Sp8 and Sp9 within the zinc-finger domain. Thus, these two proteins might bind to same genes and serve several redundant roles. Indeed, we observed that, in Sp8/Sp9 double conditional mutant OB, nearly all migratory interneurons (neuroblasts) failed to exit the RMS. Accordingly, none of mature interneurons were observed in the granule cell layer, external plexiform layer and periglomerular layer of the OB. These results suggest that Sp8 and Sp9 have central roles in OB interneuron development. Diverse molecular mechanisms underlying these phonotypes are currently under study.

Disclosures: **J. Li:** None. **C. Wang:** None. **D. Qi:** None. **Y. You:** None. **Z. Yang:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

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Topic: A.02. Postnatal Neurogenesis

Support: NCTR/FDA P00706

Title: Estrogen receptor-beta (ERβ) expression in the sexually dimorphic nucleus of preoptic area (SDN-POA) and the 3rd ventricle stem cell niche

Authors: *Z. HE, M. G. PAULE, S. A. FERGUSON;
Div. of Neurotoxicology, Natl. Ctr. For Toxicology Res., Jefferson, AR

Abstract: Perinatal exposure to estrogens leads to increased SDN-POA volumes in male and female weanling rats [He et al., 2012]. Proliferative activities of hypothalamic stem cells can be identified using Ki67 labeling within stem cell reservoirs (the 3rd ventricle stem cell niche (3VSCN) and the hypothalamic parenchyma which includes the SDN-POA [He et al., 2013a]) and indicate that stem cell activity is one of the driving forces in SDN-POA development. Here, we determined if the regulation of hypothalamic stem cell activity is mediated via an estrogen receptor-initiated pathway by examining ERβ expression in the 3VSCN and the SDN-POA. **Methods.** Hypothalamic slices containing the 3VSCN or SDN-POA from weanling (n=12) and adult (n=8) Sprague-Dawley rats were processed using a triple fluorescence labeling method: anti-ERβ (label #1); anti-calbindin D28K (CB28), -nestin, or -Ki67 (label #2) and DAPI (label #3, to delineate cellular nuclei). The SDN-POA was delineated using CB28 and DAPI since DAPI-labeling demarcates the SDN-POA similar to that of CB28 [He et al., 2013b]. The 3VSCN was delineated using nestin-immunoreactivity (ir) and adjacent slices were processed to determine whether proliferating cells (Ki67-ir positive) expressed ERβ. Traditional stem cell reservoirs such as the subventricular zone (SVZ) and subgranular zone (SGZ) served as reference or control areas. **Results.** Many cells within the SDN-POA exhibited ERβ-ir labeling which was irrespective of CB28-ir labeling. The majority of 3VSCN cells that expressed nestin also expressed ERβ. Interestingly, a few proliferating (Ki67 positive) cells within the 3VSCN and the hypothalamic parenchyma, including the SDN-POA, displayed ERβ-ir labeling. A subset of cells in the SVZ was double-labeled with nestin and ERβ and a few cells co-labeled with Ki67 and ERβ. There was little evidence that proliferating cells in the SGZ expressed ERβ. **In summary**, ERβ-ir may serve as a marker of estrogen-receptor associated pathways within the SDN-POA that subserve sexually-relevant behaviors and regulate cell regenerative cycles during proliferative periods (i.e., ERβ-Ki67 co-labeled cells). Because ERβ was heavily expressed in the 3VSCN in either nestin or Ki67-positive cells, ERβ may play a role in the development of sexual

dimorphism by regulating cellular proliferation in the sexually dimorphic structures surrounding the 3VSCN, including the SDN-POA. NCTR protocols P00710 and P00706 supported this project.

Disclosures: Z. He: None. **M.G. Paule:** None. **S.A. Ferguson:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

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Topic: A.02. Postnatal Neurogenesis

Support: DFG grant SFB400 (Germany)

Title: Sustained increase of neuronal polysialic acid level does not affect the nervous system development and maintenance but induces a mild behavioral deficit that may be attributed to synaptic dysfunction

Authors: *S. NGAMLI FEWOU[1,2], I. RÖCKLE[4], H. HILDEBRANDT[5], K. HAASTERT[5], C. GROTHE[5], V. GIESELMANN[2], M. ECKHARDT[3];
[1]Fac. of Hlth. Sciences, Univ. Des Montagn, Bangangte, Cameroon; [2]Inst. of Biochem. and molecular biology, [3]Univ. of Bonn, Bonn, Germany; [4]Neuroglycobiochemistry, [5]Univ. of Hannover medical school, Hannover, Germany

Abstract: The polysialic acid (PSA) modification of the neural cell adhesion molecule (NCAM) is an abundant posttranslational modification during development. During postnatal development, PSA is rapidly down-regulated but remains expressed in certain brain regions that are involved in neurogenesis and display structural plasticity. This significant downregulation suggests that removal of PSA after modeling of the nervous system is an important task during postnatal development of the brain. In contrast, increase of PSA level is observed in some neurological disorder including multiple sclerosis and Alzheimer's diseases. We therefore hypothesized that prevention of postnatal downregulation of PSA will affects the structural and functional properties of the nervous system. To test our hypothesis, we generated transgenic mice overexpressing the polysialyltransferase (ST8SiaIV) in neurons under the control of Th1.2 promoter. The transgene expression prevented the postnatal downregulation of PSA in neuron and most NCAM-140 and NCAM-180 in the forebrain was polysialylated. Examination of the brain region such as the olfactory bulb, lamination of the mossy fiber tract in the hippocampal formation and the development of the corticospinal tract did not revealed any abnormality as

observed in NCAM- and PSA-deficient mice. In addition normal myelin structure and myelination was observed in transgenic mice compared to wild-type littermates. In general, morphological examinations of nervous system did not reveal structural abnormalities in transgenic mice as compared to wild-type controls. Behavioral studies revealed a reduced rearing activity and exploratory behavior, while parameters of motor activity like distance traveled and mean velocity in an open field or rotarod performance were not affected in transgenic mice. These results demonstrate that preventing the postnatal downregulation of PSA has a significant effect on exploratory behavior which may be attributed to some synaptic dysfunction in the brain. The absence of a neurodevelopmental phenotype makes the transgenic mouse a useful model system suited for further exploring the effects of sustained increased of PSA level in the brain homeostasis.

Disclosures: **S. Ngamli Fewou:** None. **I. Röckle:** None. **H. Hildebrandt:** None. **K. Haastert:** None. **C. Grothe:** None. **V. Gieselmann:** None. **M. Eckhardt:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.02. Postnatal Neurogenesis

Title: Diazepam Binding Inhibitor (DBI) regulates the hippocampal stem cell pool

Authors: I. G. DUMITRU[1,2], A. NEITZ[1,2], J. ALFONSO[1,2], *H. MONYER[1,2];
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Abstract: In the mammalian brain there are two areas where neurogenesis persists in the adult: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus. Diazepam Binding Inhibitor (DBI) is expressed by SVZ neural progenitors, and plays an important role in regulating adult SVZ neurogenesis by modulating the GABA regulatory signal (Alfonso et al., 2012). Here we present evidence that DBI is strongly expressed also in SGZ stem cells and its binding to the benzodiazepine binding site of the GABAA receptor leads to a reduction in GABA-induced currents. *In vivo* knockdown of DBI in the dentate gyrus via a lentiviral strategy reduces the stem cell and amplifying progenitor population, while the overexpression had the opposite effect. Our results indicate that DBI acts at the level of the SGZ stem cells and amplifying progenitors and promotes their proliferation at the expense of their differentiation. Furthermore, knocking down DBI in the dentate gyrus abolished the proliferative

effect of enriched environment, indicating that DBI might play a role in regulating the influence of external factors on the SGZ stem cell niche. In conclusion, our results suggest that DBI regulates the production of both inhibitory (SVZ) and excitatory neurons (SGZ) and modulates the environmentally induced changes of the niche dynamics.

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Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.02. Postnatal Neurogenesis

Support: Dedicated Health Research Funds of the University of New Mexico School of Medicine

PREP Grant R25 GM075149 (NIH/NIGMS)

Title: Synaptosomal Associated Protein-25 (SNAP-25) is not essential for the survival and morphological maturation of newborn dentate granule cells in adult hippocampus

Authors: *K. C. GUSTUS[1], L. LI[1], Y. GU[2], S. GE[2], L. CUNNINGHAM[1], M. C. WILSON[1]; [1]Neurosciences, Univ. of New Mexico, Albuquerque, NM; [2]Dept. of Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Adult hippocampal neurogenesis is critical for cognition and behavior, but the requirements for integration of immature neurons into a pre-existing mature dentate circuit have not been fully elucidated. In contrast to the widely held view that the survival and maintenance of neurons in the developing CNS is dependent on their ability for action potential (AP)-dependent synaptic communication, more recent studies have demonstrated considerable brain development in the absence of evoked neurotransmitter release. However, the role of AP-dependent synaptic transmission by adult-generated dentate granule cells (DGCs) for their survival within the existing circuitry has not been elucidated. Here, we utilized a unique SNAP-25 $^{fl/fl}$:Rosa dTomato bi-transgenic mouse to selectively inactivate AP-dependent synaptic transmission in adult hippocampal progenitors using retroviral delivery of Cre recombinase. SNAP-25 is a constituent of the presynaptic exocytosis complex that is specifically responsible for evoked synaptic transmission, but not for AP-independent, spontaneous neurotransmitter release. For these studies, retrovirus was stereotaxically delivered to adult hippocampal

progenitors in adult SNAP-25 $^{fl/fl}$:Rosa dTomato bi-transgenic mice or SNAP-25 $^{wt/wt}$:Rosa dTomato control mice, targeting the hilus/subgranular zone of the dorsal and ventral hippocampal dentate gyrus. Mice were sacrificed at 4 or 8 weeks post retroviral injection and dTomato+ DGCs were imaged using confocal microscopy. Adult generated dTomato+ DGCs were observed in both SNAP-25 $^{fl/fl}$:Rosa dTomato and control mice. Interestingly, SNAP-25 gene inactivation had no overt impact on dendritic or axonal growth by newborn DGCs. Newborn DGCs from both genotypes extended dendrites into the molecular layer and mossy fiber axons to the CA3/CA2 border. These initial studies suggest that AP-dependent neurotransmitter release by newborn DGCs is not essential for their survival or morphological integration into the adult hippocampal circuitry. Whether SNAP-25$^{-/-}$ newborn DGCs display more subtle deficits in dendritic and axonal complexity is currently under investigation.

Disclosures: **K.C. Gustus:** None. **L. Li:** None. **Y. Gu:** None. **S. Ge:** None. **L. Cunningham:** None. **M.C. Wilson:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

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Title: β1-integrin and BMP pathway interactions in the regulation of the adult hippocampal stem cell niche

Authors: ***S. M. BROOKER**, A. M. BOND, C.-Y. PENG, J. A. KESSLER;
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Abstract: In the adult mammalian brain, neurogenesis persists throughout life in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. Ongoing hippocampal neurogenesis has been shown to play a key role in cognition and mood regulation. Diverse signaling molecules regulate the adult NSC

niche, including components of the extracellular matrix. Here we investigated the role of the extracellular matrix-interacting protein β1-integrin in regulating neural stem cell maintenance and fate commitment in the adult DG. We further examined how β1-integrin interacts with the bone morphogenetic protein (BMP) signaling pathway in adult NSCs. We used the Cre/LoxP system to genetically ablate β1-integrin in the dentate gyrus of adult β1-integrin floxed mice or from cultured NSCs derived from these mice. We also performed co-immunoprecipitation studies to investigate the interaction between β1-integrin and the BMP receptor subunits. Our results show that genetic ablation of β1-integrin in NSCs *in vitro* leads to a decrease in levels of stem and progenitor cell proliferation, as well as an increase in astrocytic differentiation, an effect which can be reversed by the addition of the BMP signaling inhibitor noggin. Conditional knockout of β1-integrin in the DG of adult mice leads to disruption of the structural integrity of the SGZ and granule cell layer. Knockout of β1-integrin in the DG also alters the size of the NSC pool and modulates cell fate commitment. Further, genetic ablation of β1-integrin in NSCs leads to an increase in overall levels of BMP signaling and β1-integrin physically interacts with the type I BMP receptors in both the wild type adult hippocampus and in cultured NSCs. We conclude that β1-integrin plays a dual role in the adult hippocampal niche, both maintaining the structural integrity of the DG and regulating NSC fate commitment via modulation of BMP signaling.

Disclosures: **S.M. Brooker:** None. **A.M. Bond:** None. **C. Peng:** None. **J.A. Kessler:** None.

Poster

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Topic: A.02. Postnatal Neurogenesis

Support: ANR Grant SynD2

Title: The transcription factor NeuroD2 regulates synaptic integration in the postnatal olfactory bulb

Authors: ***S. BUGEON**[1], O. HARDT[2], A. BOSIO[2], H. CREMER[1], A. DE CHEVIGNY[1]; [1]Developmental Biol. Inst. of Marseille, Marseille Cedex 09, France; [2]Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Abstract: Integration of new neurons into an existing circuitry represents a prerequisite for effective cell therapy in CNS disease and trauma. Postnatal olfactory neurogenesis is a valid

system to study the molecular control of this process. Using a genetic screen to isolate genes whose expression peaks concomitantly with synaptogenesis of new neurons in the olfactory bulb, we identified the transcription factor NeuroD2 as a candidate regulator of synaptic integration in the postnatal olfactory bulb. Using mutant mice in concert with transplantation approaches and *in vivo* brain electroporation we studied NeuroD2 function in the OB. We find that in mice constitutively lacking NeuroD2 olfactory bulb interneurons show normal migration, integration and dendritic spine formation. However, in a competitive context, created either by transplantation of mutant cells into a WT environment or via sh-RNA induced knock-down, NeuroD2 loss-of-function leads to a strong decrease in synapse number of postnatally generated interneurons. These results suggest that inter-cellular differences of NeuroD2 expression between neurons, more than absolute levels of NeuroD2, impact on synaptic integration.

Disclosures: S. Bugeon: None. **O. Hardt:** None. **A. Bosio:** None. **H. Cremer:** None. **A. de Chevigny:** None.

Poster

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Topic: A.02. Postnatal Neurogenesis

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Activity-dependent regulation of neurogenesis after stroke

Authors: *H. LIANG, S. T. CARMICHAEL;
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Abstract: Stroke is the leading cause of death in America and a leading cause of adult disability. Post-stroke neurogenesis has been implicated in repair and functional recovery in brain injury; however the cellular and molecular mechanism that regulates neurogenesis after stroke remains unclear. Although activity has been shown to regulate neurogenesis during development in normal brain, how modulation of activity affects neurogenesis after stroke is not fully elucidated. In this study, we employed forced use of the paretic forelimb that mimics constraint-induced movement therapy (CIMT) as a physical modulation of activity after stroke. We found that forced use of forelimb that was associated with the stroke affected area enhanced neuroblast migration and neural progenitor proliferation in the peri-infarct cortex on post-stroke day 14 and neuronal differentiation was increased on post-stroke day 60. Interestingly, when forced use was

performed in the hindlimb that was associated with stroke non-affected motor cortex from the ipsilateral hemisphere, neurogenesis in peri-infarct cortex was largely abolished. These data suggested that activity-induced neurogenesis after stroke is circuit specific and is dependent on regions of modulation. To further demonstrate the role of activity in a cell type specific manner, we utilized the Designer Receptors Exclusively Activated by Designer Drugs (DREADD) as a pharmacological approach to modulate neuronal activity. Preliminary data suggests that activation of the CaMKII-expressing neurons in the peri-infarct cortex increased neurogenesis 14 days after stroke.

Disclosures: H. Liang: None. **S.T. Carmichael:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.02. Postnatal Neurogenesis

Support: FONDECYT 1150933

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Title: The Wnt receptor Frizzled-1 regulates neurogenesis in the adult hippocampus

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Abstract: In the adult hippocampus, new neurons are continuously generated from neural progenitor cells (NPCs) that reside in the subgranular zone of the dentate gyrus. This process is controlled by Wnts, a family of 19 secreted glycoproteins that bind to Frizzled (FZD) receptors and co-receptors to trigger the canonical Wnt/beta-catenin pathway or non-canonical Wnt signaling cascades. Wnts regulate different steps of adult neurogenesis such as proliferation and differentiation of NPCs, and maturation of newborn neurons. The regulation of the sequential steps of neurogenesis might be mediated by specific Wnt receptors. Here, we studied the potential role of FZD1 in the regulation of adult hippocampal neurogenesis. To evaluate *in vivo* the role of this receptor, we targeted FZD1 in newborn cells using retroviral-mediated RNA interference. Retroviruses expressing control or FZD1 shRNAs were injected stereotaxically into

the dentate gyrus of 2-month-old mice. First, we evaluated neuronal fate commitment, and determined that FZD1 knockdown resulted in a strong decrease in the differentiation of newborn cells into neurons. In cells that were able to differentiate into neurons, we evaluated migration within the granule cell layer (GCL). Adult-born neurons remain primarily positioned within the inner third of the GCL; in agreement, we observed that newborn neurons expressing control shRNA were located in the first third of the GCL. On the other hand, FZD1-deficient cells showed an extended migration and were also located in the middle and outer third of the GCL. Finally, no differences were observed in dendrite development between control and FZD1-deficient newborn granule neurons. Altogether, these results indicate that FZD1 regulates specific stages of neurogenesis being involved in neuronal differentiation and migration of newborn neurons.

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Poster

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Title: The role of post-translational modification of FOXP2 in brain development

Authors: ***N. USUI**[1], M. CO[1], M. HARPER[1], M. A. RIEGER[2], J. D. DOUGHERTY[2], G. KONOPKA[1];
[1]Dept. of Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [2]Dept. of Genet. and Dept. of Psychiatry, Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Mutations in the gene encoding forkhead box P2, FOXP2 result in brain developmental phenotypes including altered cerebellar size in both human patients and rodent models. FOXP2 is a transcription factor expressed in the brain; however, the regional contribution of FOXP2-mediated signaling to brain development and function remains mostly uncharacterized. In addition, while prior studies have demonstrated an important role of post-translational modifications (PTMs) in regulating the function of transcription factors in the central nervous system (CNS), the importance of PTMs of FOXP2 is unknown. We uncovered that FOXP2 is sumoylated during brain development, and identified a consensus site responsible for FOXP2 sumoylation, which is evolutionally conserved from mouse to human. Furthermore, PIAS3 and SUMO-1/2 were also identified as the E3 SUMO ligase and SUMO proteins involved in the reaction of sumoylation respectively. To investigate the functional role of FOXP2 sumoylation, we overexpressed FOXP2 wild-type (WT) and a non-sumoylated mutant of FOXP2 (FOXP2 KR) in mouse neuronal progenitors. This resulted in neurite outgrowth being promoted by FOXP2 WT, but not FOXP2 KR. In addition, gene regulation by FOXP2 was also regulated by the sumoylation state of FOXP2. Taken together, sumoylation of FOXP2 has important roles in neuronal development and regulation of downstream targets of FOXP2.

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Topic: A.02. Postnatal Neurogenesis

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Title: Granule neuron progenitors in the developing murine cerebellum exhibit asymmetric cell division

Authors: *A. CHATTERJEE[1], P. HALDIPUR[2], I. SIVAPRAKASAM[1], V. BABU[2], S. GOVINDAN[1], S. MANI[1,2];
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Abstract: Granule neurons found in the cerebellum make for the largest population of neurons in the human and murine brain. They are generated postnatally in the External Granule Layer (EGL) wherein granule neuron progenitors undergo extensive number of cell divisions. These progenitors subsequently exit the cell cycle and migrate ventrally to form the Inner Granule Layer (IGL) where they differentiate into mature granule neurons. However, it is not known whether granule progenitors exhibit bias in the orientation planes of cell division or they divide randomly. A biased orientation might imply sensing of directional cues by the progenitors either from the Purkinje cells ventrally or the pia dorsally. We found there to be an increase in number of divisions parallel to the pia from P5-P14 (BALB/c mice used for the study). We investigated the role of the classical mitogen Sonic hedgehog (Shh), which is secreted by Purkinje cells, in regulating the division plane. Significant number of granule progenitors underwent parallel divisions in cyclopamine (Smoothened antagonist) treated mice as opposed to more number of perpendicular divisions in SAG (Smoothened agonist) treated mice. This shows that Shh can regulate plane of division in granule progenitors. Moreover, on perturbation of Shh levels using cyclopamine it was seen that number of cells expressing NeuroD1 and β-III-tubulin increased while number of PCNA positive cells decreased in the EGL. The converse was noticed in mice treated with SAG. β-catenin is a putative cell fate determinant and has been causally implicated in maintaining cerebral and cerebellar ventricular zone progenitors in cell cycle. We checked for possible asymmetric distribution of β-catenin during progenitor divisions and observed increasing asymmetric distribution of the protein over the course of cerebellar development. Additionally, β-catenin was always found to be expressed more in the daughter cell in contact with printed Shh stripes. These results show that the general principles regulating progenitor expansion and neurogenesis might be conserved across different developing regions of the brain.

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Poster

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Topic: A.02. Postnatal Neurogenesis

Support: Stockton University REU Grant

Title: The Akt-mtor pathway is a key regulator of neurogenesis in the subventricular zone

Authors: *G. PEZZANO, M. A. FINGER, J. MERCURIO, N. W. HARTMAN;
Sch. of Natural Sci. and Mathematics, Stockton Univ., Galloway, NJ

Abstract: The Akt-mTOR pathway is important for cellular growth, proliferation and differentiation. Dysregulation of this pathway in neural stem cells (NSCs) can lead to neurodevelopmental pathologies, such as Tuberous Sclerosis. It is unclear whether Akt and mTOR play roles independent of each other in NSC development. NSCs in the subventricular zone (SVZ) continually generate new daughter cells that migrate to the olfactory bulb (OB), differentiating into neurons. Here, we show that a constitutively active form of Akt resulted in a threefold increase in the number of newly born neurons in the OB. In contrast to driving mTOR alone, Akt activation did not result in any apparent aberrant migration in the SVZ or OB. Both Akt and mTOR activation resulted in increased dendrite length and complexity. We also observed that Akt induces proliferation of cells in the SVZ independent of mTOR activation. These data suggest that Akt controls NSC differentiation via mTOR but may exert proliferative effects independently.

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Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.02. Postnatal Neurogenesis

Support: NIH Pharmacological Sciences Training Grant (T32GM007040-39)

Title: Regulation of adult neurogenesis by the hippocampal cholecystokinin network

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Abstract: Adult neurogenesis is a unique and poorly understood form of neuroplasticity that in humans is essentially restricted to the dentate gyrus (DG) of the hippocampus. Unlike developmental neurogenesis, this enigmatic process is tightly regulated by the activity of local neuronal circuits and afferent projecting systems. The identity of specific cell types, neurotransmitters, and receptors that facilitate this regulation remains critically understudied. Studies utilizing animal and cell culture models suggest that the neuropeptide cholecystokinin

(CCK) serves as a survival signals for neural stem cells in the adult brain. We have therefore examined the hypothesis that CCK may regulate adult neurogenesis by promoting neural stem cell proliferation and asymmetric neuronal fate specification, neuronal progenitor survival, and integration of immature neurons into the surrounding circuitry. We have found that neural stem cells express mRNA for the Gq-coupled CCK2 receptor, and exhibit Ca2+ transients following stimulation with the CCK2-receptor selective form of CCK (CCK8) which can be blocked by the CCK2R antagonist YM022. Intravenous administration of CCK8 produces an increase in proliferating cells in the DG, and we have found that *in vivo* chemogenetic stimulation of CCK-releasing neurons in the DG produces an increase in stem cell proliferation. Moving forward we will identify whether this effect can be blocked by knocking down CCK synthesis. To further clarify the role of the CCK-network in activity-dependent adult neurogenesis, we will utilize retrograde transynaptic tracing to identify interacting local and distant and neuronal populations.

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Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.02. Postnatal Neurogenesis

Title: CPEB4 regulates olfactory experience-dependent granule cell survival in the early postnatal olfactory bulbs

Authors: C.-S. TSENG[1,2], *Y.-S. HUANG[1,2];
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Abstract: Cytoplasmic polyadenylation element binding protein 4 (CPEB4) is a sequence-specific RNA-binding protein, which promotes polyadenylation-induced translation of target mRNAs. Although CPEB4 is distributed widely in the brain, the neuronal function of CPEB4 remains to be revealed. In this study, we found that 3-month-old CPEB4 knockout (KO) mice had smaller olfactory bulbs (OBs), in which the granule cell layer was significantly reduced. CPEB4 null mice with normal olfactory sensitivity and memory displayed impaired olfactory discrimination, which may result from the reduction of granule cells. Since OBs continue to replenish with new interneurons from adult neurogenesis to maintain its size, we first examined if any defect in this process. Unexpectedly, no difference in adult neurogenesis between wild-type (WT) and KO littermates was found. Instead, CPEB4 deficiency-induced OB hypoplasia

resulted from increased apoptosis in granule cells during the early (i.e. the first two weeks) postnatal OB development. Moreover, sensory deprivation by naris occlusion enhanced granule cell apoptosis and reduced OB size in WT but not CPEB4 KO mice, indicating that CPEB4 governs OB growth in an olfactory experience-dependent manner. Furthermore, using RNA immunoprecipitation coupled with microarray, several RNA candidates bound by CPEB4 were identified. Currently, we are investigating which RNA targets are indeed translationally regulated by CPEB4 to contribute to the granule cell survival during OB development.

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Poster

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Title: L-type voltage gated calcium channels Cav1.2 and Cav1.3 in adult neurogenesis

Authors: B. VOELKENING[1], T. WEBER[1,2], *D. BARTSCH[1];
[1]CIMH and Med. Fac. Mannheim, Heidelberg Univ., Mannheim, Germany; [2]AHG Klinik Wilhelmsheim, Oppenweiler, Germany

Abstract: Differentiation of adult neural progenitor cells (NPCs) into neurons is induced by a proneural gene expression program via electrical stimuli. NPCs directly sense these excitatory changes via L-type voltage gated calcium channels (LVGCC) and translate them into Ca2+-influx triggering cascade of gene expression. LVGCC Cav1.2 and Cav1.3 are expressed in the brain, but their expression and function in adult NPCs remains elusive due to a lack of specific antibodies or selective pharmacological tools. To define the role of LVGCCs in the dentate gyrus (DG) in neurogenesis, we used a transgenic mice mouse model with conditional, inducible knockout of alpha subunits (Cacna1c and Cacna1d) of Cav1.2 and Cav1.3 channels specifically in type-1 cells of the DG (directed by GLAST-CreERT2). To trace cells with ablated Cacna1c or Cacna1d genes and their progeny, we included in the mouse model a Cre inducible GFP transgene (GFPflox) which mirrors the deletion of the conditional Cacna1c and Cacna1d -alleles by GFP expression. We induced ablation of Cacna1c and Cacna1d in 8 weeks old mice and

compared the GFP expression to control mice (GLAST-CreERT2/GFPflox). The total amount of GFP+ cells was counted, along with the neuronal marker NeuN at 6 months of age. We found a significant decrease in GFP+ and GFP+/NeuN+ cells in both Cav1.2-/- and Cav1.3-/- mice in the DG, while the ratio of GFP+/NeuN+ neurons to all GFP+ cells did not differ compared to controls. Next, we dissected the hippocampus of adult mice, isolated the GFP positive cells by FACS few days after tamoxifen-induced LVGCCs ablation and established NPC lines. Likewise to results seen *in vivo*, both Cav1.2-/- and Cav1.3-/- NPC cells *in vitro* show much lower proliferation compared to control NPCs. These findings together show that the differentiation into adult neurons is not generally impaired, but rather a reduction of the precursor cell population occurs in the LVGCC knockouts. Our results indicate that ablation of both Cacna1c and Cacna1d in Type1 cells affect their proliferation *in vivo* and *in vitro*. Interestingly, this defect in both conditional mutants can be reversed by electroconvulsive seizures (ECS) *in vivo*, the mouse equivalent of electroconvulsive therapy (ECT) in humans. Studies to investigate this effect *in vitro* are in progress. Our results suggest an eminent role of LVGCCs on adult neurogenesis and will provide a novel insight into their effect on survival, proliferation and differentiation of NPCs.

Disclosures: **B. Voelkening:** None. **T. Weber:** None. **D. Bartsch:** None.

Poster

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Title: Down regulated necdin expression triggers proliferation of postmitotic neurons of the rat cortex

Authors: *S. LIU, R. LIU, H. QUE, J. YANG, Q. LIN, Y. LIU, S. JING, S. JING;
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Abstract: Mature neurons have long being regarded as terminally differentiated cells and therefore can no longer enter the cell cycle or proliferate. Quite a number of the early reports showing evidence of adult animal brain neurogenesis are now believed to be actually a view of neurons derived from neural stem cells or neural progenitor cells. NeuN-positive neurons at layer VI of the cortex of adult rat were thought to be cells newly differentiated from stem cells in the

periventricle area. Despite of these facts, we found that the reduced level of necdin, which can be induced by either the tri-iodo-L-thyronine (T3) or the necdin RNAi treatment, is able to trigger the proliferation of primary neurons both *in vitro* and *in situ* of the adult rat cortex. The enriched primary neurons (purity up to 99.99%) were first treated with T3 at different concentrations. Substantial amount of dividing neurons at different mitosis phases were observed, Low dose of T3 made parvocellular neuron division and the high concentration of T3 resulted in magnocellular neuron proliferation. About 4.6±0.7% ShcC labeled primary neurons were analyzed at G2M with flow cytometry. We further demonstrated that the dividing neurons possess several characteristics of postmitotic neurons, evidenced by specific markers, electrophysiology, ultrastructural and typical synapses between the dividing neurons and others. When different concentration of T3 was reversely administrated into the cortex of adult rat by microdialysis, the substantial amount neurons *in situ* around the microdialysis areas were found to divide. The dividing neurons at different dividing phases were labeled by either both MAP2 and ShcC or MAP2 and NeuN. Further investigation suggested that T3 functions through the inhibition of necdin's expression, which leads to the alteration of the subcellular localization of E2F1 and ultimately triggers the expression of cyclin. This phenomena is further confirmed by necdin RNAi, during which the reduced expression of necdin triggers the division of several kinds of neurons *in vivo*. To be specifically, Necdin-RNAi-adenoviruses were injected into the layer II-V of the cortex, and a substantial amount of neurons at different mitosis phases, immunolabeled by either NeuN, ShcC or Map2 were observed and recorded around the injected areas. Even the pyramidal neurons labeled by NeuN and MAP2 at layer III and V were observed to divide. Postmitotic neurons still possess the ability for proliferation provides us an opportunity in neural regeneration and thereof, renews the hope in developing strategies for cure of neural degenerative diseases.

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Poster

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Title: Gmip-mediated inactivation of RhoA controls speed of neuronal migration in the postnatal mouse brain

Authors: *H. OTA[1,2], T. HIKITA[1], M. SAWADA[1], T. NISHIOKA[3], M. MATSUMOTO[1], M. KOMURA[1], A. OHNO[1], Y. KAMIYA[1], T. MIYAMOTO[1], N. ASAI[4], A. ENOMOTO[4], M. TAKAHASHI[4], K. KAIBUCHI[3], K. SOBUE[2], K. SAWAMOTO[1];
[1]Dept. of Developmental and Regenerative Biol., [2]Dept. of Anesthesiol. and Intensive Care Med., Nagoya City Univ. Grad. Sch. of Med. Sci., Nagoya-City, Japan; [3]Dept. of Cell Pharmacol., [4]Dept. of Pathology, Nagoya Univ. Grad. Sch. of Med., Nagoya-City, Japan

Abstract: In the postnatal mammalian brain, neural stem cells continuously generate new neurons in the ventricular-subventricular zone (V-SVZ). New neurons born in the rodent V-SVZ take the long journey to the olfactory bulb (OB), where they differentiate into interneurons. The intracellular mechanisms that precisely control the neurons' migration speed, enabling their well-organized movement, remain unclear. In this study, we performed a global proteomic search for proteins interacting with Girdin, an essential protein for postnatal neuronal migration, by combination of immunoprecipitation and liquid chromatography tandem mass spectrometry (LCMS/MS), and we identified Gem-interacting protein (Gmip), a RhoA-specific GTPase-activating protein, as a key factor in saltatory neuronal migration. Rho signalling is known to affect the morphology and movement of various cell types, including neurons. Using RNA interference (RNAi) and FRET imaging, we revealed that RhoA is activated at the proximal leading process of migrating neurons, where Gmip is also localized and negatively regulates RhoA. Gmip controls the saltatory movement of neurons that regulate their migration speed and 'stop' positions in the olfactory bulb, thereby altering the neural circuitry. This study demonstrates that Gmip serves as a brake for the RhoA-mediated movement of neuronal somata, and highlights the significance of speed control in the well-organized neuronal migration and the maintenance of neuronal circuits in the postnatal brain.

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Poster

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Title: Genetic deletion of intracellular fibroblast growth factor 14 (fgf14) disrupts transition of late immature to mature newly born granule neurons in the adult dentate gyrus

Authors: *M. A. ALSHAMMARI[1,2], T. K. ALSHAMMARI[1,2], F. SCALA[1], M. N. NENOV[1], F. LAEZZA[1];
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Abstract: Growing evidence indicates that adult neurogenesis, the production of mature neurons from progenitor cells in the adult mammalian brain, is linked to the etiology of neurodegenerative and psychiatric disorders. However, a complete understanding of the molecular elements at the base of adult neurogenesis remains elusive. Here, we provide evidence for a previously undescribed function of fibroblast growth factor 14 (FGF14), a brain disease-associated factor that controls neuronal excitability and synaptic plasticity, in regulating adult neurogenesis in the dentate gyrus (DG). Through a combination of BrdU incorporation studies and confocal imaging, we show that FGF14 is dynamically expressed in DG at various developing states of neural progenitors. Genetic deletion of *Fgf14* in *Fgf14*$^{-/-}$ mice leads to a significant increase in the late immature and early mature population of doublecortin and calretinin positive neurons, while the number of early progenitor Sox2 positive stem cells and mature calbindin positive neurons remained constant. Caspase-3 activity is unaffected by deletion of *Fgf14* ruling out reduced survival as a major cause of the deficit. Ongoing studies are evaluating the impact of *Fgf14* genetic deletion in the functional integration of newly born neurons in the DG circuitry. Our results provide evidence for a novel signaling pathway associated with FGF14 expression controlling adult neurogenesis, providing insights into the biology of complex brain disorders

Disclosures: **M.A. Alshammari:** None. **T.K. Alshammari:** None. **F. Scala:** None. **M.N. Nenov:** None. **F. Laezza:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 663.25/A100

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant NS050338

University of Connecticut

Title: EphA4-ephrin signaling regulates postnatal development of the rostral migratory stream

Authors: *M. EASTMAN, K. L. BAKER, N. B. GALLO, J. C. CONOVER;
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Abstract: The rostral migratory stream (RMS), the migratory pathway for neuronal precursors to transit through the mouse anterior forebrain from the subventricular zone to the olfactory bulb, undergoes significant cytoarchitectural rearrangement during the first few weeks of postnatal life. During this time, an astroglial meshwork is established to control neuronal precursor migration, restricting migrating cells to a tightly defined area via cell-cell contact-dependent signaling. Disruption of the postnatal astroglial meshwork can lead to atypical neuroblast orientation and escape from the RMS. Little is known about the molecular coordinators of RMS development, glial meshwork formation, or migratory control of neuroblasts. Receptor tyrosine kinase Ephs and their membrane-bound ephrin ligands are indispensable for normal embryonic development and have been shown to persist in regulating adult stem cell niches. Here, we provide evidence for the expression of EphA4 and its multiple ligand ephrins in the RMS from postnatal development through adulthood, and show that disruption of EphA4 intracellular signaling impairs the formation of the astroglial meshwork and neuronal precursor confinement within the RMS. Based on our studies, we propose a model for development of the postnatal RMS into the mature, adult RMS, and implicate EphA4/ephrin molecular mechanisms in regulating tangential neuronal precursor migration in the mature brain.

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

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 663.26/A101

Topic: A.02. Postnatal Neurogenesis

Support: Wellcome trust

MRC

Title: CHD7 fine-tunes Reelin expression in cerebellar neuron progenitors by remodeling chromatin structure

Authors: *D. WHITTAKER[1,2], K. L. H. RIEGMAN[3], B. PIJUAN SALA[3], H. HEBAISHI[4], S. KASAH[3], T. YU[3], A. CARUSO[5], A. MARQUES[6], C. MICHETTI[5], A. SHAH[3], W.-W. TEE[7], D. REINBERG[7], C. PONTING[6], M. SCATTONI[5], F. WARDLE[8], H. VOLK[2], I. MCGONNELL[2], C. FERNANDES[9], M. A. BASSON[3];
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Abstract: Mutations of the ATP dependent chromatin-remodeling factor CHD7 are the major cause of CHARGE syndrome (Coloboma, Heart defects, Atresia of the choanae, Retarded growth and development, Genital-urinary anomalies and Ear defects). Symptoms attributable to neurodevelopmental defects including developmental delay, coordination problems and autistic traits are frequently identified. Furthermore we recently reported cerebellar vermis hypoplasia and abnormal foliation by magnetic resonance imaging (MRI) in a subset of patients. These neuroanatomical findings imply a role for CHD7 in post-natal cerebellar development, however, despite the frequency of clinical signs, mechanisms underlying neuropathology in patients remain unknown. Here, we report cerebellar hypoplasia, purkinje cell disorganization, motor deficits and developmental delay in a mouse model that conditionally deletes *Chd7* from granule cell precursors (GCps) and show that Chd7 is critical in regulating granule cell proliferation and differentiation *in vivo*. We report a crucial role for Chd7 in the regulation of *Reln* gene expression and identify a significant down regulation of *Reln* in GCps of the conditional mutants by RNA-seq and qPCR. Recessive mutations in RELN are associated with cerebellar hypoplasia and expression is down regulated in post-mortem cerebella from autistic patients. Despite these findings mechanisms that regulate RELN expression are not known. We provide functional evidence that Reln contributes to cerebellar hypoplasia *in vivo* by showing a partial rescue of the central lobule hypoplasia, which was most severely affected in the *Chd7* mutant, through the

ectopic expression of *Reln*. Finally, we report a mechanistic link between Chd7 and Reln and show that Chd7 is responsible for maintaining an open chromatin state at the Reln promoter. These findings outline a critical role for CHD7 in fine-tuning gene expression in the postnatal cerebellum and provide a link between CHD7 and a pathway associated with cerebellar hypoplasia, motor coordination deficits and autism.

Disclosures: **D. Whittaker:** None. **K.L.H. Riegman:** None. **B. Pijuan Sala:** None. **H. Hebaishi:** None. **S. Kasah:** None. **T. Yu:** None. **A. Caruso:** None. **A. Marques:** None. **C. Michetti:** None. **A. Shah:** None. **W. Tee:** None. **D. Reinberg:** None. **C. Ponting:** None. **M. Scattoni:** None. **F. Wardle:** None. **H. Volk:** None. **I. McGonnell:** None. **C. Fernandes:** None. **M.A. Basson:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 663.27/A102

Topic: A.02. Postnatal Neurogenesis

Title: The role of camkiiα-expressing granule cells in the adult olfactory bulb

Authors: ***S. MALVAUT**[1], L. DAROLES[2], L. DAVID[1], S. GRIBAUDO[2], I. CAILLÉ[2], A. SAGHATELYAN[1];
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Abstract: The olfactory bulb (OB) is one of few brain regions that constantly renews its interneuronal populations, periglomerular and granule cells (GCs). GCs play an important role in the odor information processing and different subtypes of GCs have been described through expression of neurochemical markers. It remains however completely unknown if different GCs subtypes play a distinct role in olfactory processing and odor behaviour. We demonstrate that 50% of both pre-existing and adult-born GCs express the Ca2+/calmodulin-dependent protein kinase IIα (CaMKIIα), independently of their age. CaMKIIα is a major actor of synaptic plasticity with numerous substrates. Morphological analysis of CaMKIIα immmunoreactive cells show that they are not distinguishable from their CaMKIIα negative counterparts in terms of localization in the granule cell layer, dendritic arborization and spine density. Intriguingly, however, 90% of the early immediate gene cfos expressing cells in the OB belonged to the CaMKIIα immunoreactive population. CaMKIIα positive cells receive less inhibition as compared to their CaMKIIα negative counterparts since displaying a lower amplitude of

spontaneous and miniature inhibitory postsynaptic currents (IPSCs). The smaller inhibitory drive onto the CaMKIIα positive cells could explain the preferential recruitment of this subpopulation of GCs in basal olfactory conditions. By contrast, associative odor memory and perceptual learning paradigms resulted in the increased percentage of cfos+/CaMKIIα- cells. Pharmacogenic inhibition of CaMKIIα immunoreactive GCs using DREADD approach affected the short-term memory of animals to odors. Our data suggest that CaMKIIα positive and negative GCs may be differentially recruited and play distinct roles during different odor tasks.

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Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.02. Postnatal Neurogenesis

Support: NJ Governor's Council for Medical Research and Treatment of Autism 10-407-SCH-E-0

Title: Engrailed-2 plays a cell autonomous role in regulating proliferation and cell death in hippocampal neural stem cells *in vitro*

Authors: *M. DURENS[1], S. CHUNG[2], E. DICICCO-BLOOM[2];
[1]Rutgers Univ., Piscataway, NJ; [2]Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Engrailed-2 (En2) is a homeodomain transcription factor that plays important roles in cerebellar development. En2 knockout (KO) mice display abnormalities in cerebellar size and foliation, and we have previously shown that En2 regulates the cell cycle in cerebellar granule precursors. However, our recent behavioral studies indicate abnormalities in forebrain-related behaviors in En2-KO mice, including deficits in social interaction, fear conditioning and learning. These deficits are accompanied by changes in forebrain structure, including reduced weight, size and cell number in several areas including hippocampus. Indeed, in previous En2-KO studies at P21 we found 17% less granule neurons in dentate gyrus, accompanied by 2-fold increase in stem cell proliferation and 70% increase in immature neuron apoptosis. These abnormalities in neurogenesis have been associated with deficiencies in monoamine levels and innervation in the KO hippocampus, consistent with non cell autonomous mechanisms. On the other hand, while major En2 expression is in the hindbrain, we have found low levels of En2

expression in the hippocampus, raising the possibility of cell autonomous functions. To examine this issue, neurosphere cultures were derived from hippocampus of wild type (WT) and KO mice. Expression of En2 mRNA was detected in WT neurospheres but was absent in KO cells. To examine cell autonomous effects, primary neurospheres from P7 WT and KO mice were incubated in defined media with FGF and EGF and assayed for neurosphere numbers and markers of proliferation (BrdU incorporation) and apoptosis (cleaved-caspase3, pyknosis). The assay revealed no change in numbers of primary spheres, but showed an increase in the frequency of larger spheres in En2-KO, suggesting potential effects on proliferation and/or survival. Compared to WT neurospheres, the KO exhibited a 2-fold increase in BrdU labeling ($p<0.005$) as well as apoptosis ($p<0.05$). We are examining whether proliferation and apoptosis are associated with early (Sox2, Tbr2) and late (Dcx) neural stem cells respectively. These studies suggest that En2 expression plays a role in regulating proliferation and apoptosis in hippocampal neural stem cells *in vitro*. While evidence of En2 expression in hippocampal subgranular zone cells *in vivo* remains uncertain, these *in vitro* studies support a functional role. In turn, the structural and behavioral deficits observed in the KO may reflect absence of local En2 expression, though further studies are warranted. More broadly, these studies indicate how developmental regulatory genes can impact brain structure and function through diverse region and time dependent activities.

Disclosures: M. Durens: None. **S. Chung:** None. **E. DiCicco-Bloom:** None.

Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 663.29/A104

Topic: A.02. Postnatal Neurogenesis

Title: AP2gamma regulates post-natal glutamatergic neurogenesis and modulates emotional and cognitive function

Authors: N. D. ALVES[1,2], A. MATEUS-PINHEIRO[1,2], P. PATRÍCIO[1,2], E. CAMPOS[1,2], A. R. MACHADO-SANTOS[1,2], J. SILVA[1,2], V. SARDINHA[1,2], J. OLIVEIRA[1,2], J. NINKOVIC[3,4], N. SOUSA[1,2], *L. PINTO[1,2];
[1]Life and Hlth. Sci. Res. Inst. - Sch. of Hlth. Sci. - UM, Braga, Portugal; [2]ICVS/3B's – PT Government Associate Lab., Braga/Guimarães, Portugal; [3]Inst. for Stem Cell Research, Helmholtz Ctr. Munich German Res. Ctr. for Envrn. Hlth. (GmbH), Neuherberg, Germany; [4]Physiological Genomics, Med. Faculty, Univ. of Munich, Munich, Germany

Abstract: Adult neurogenesis represents one form of non-synaptic neural plasticity critical for brain homeostasis and for adaptations to the ever-changing environment. In fact, several studies suggest a relevant role of newborn neurons in the adult brain for the pathogenesis and treatment of stress-related disorders including depression. Despite its relevance for both the physiological and pathological conditions, the molecular signature behind the neurogenic process occurring in the dentate gyrus of the adult hippocampus is still poorly described. Hence, it became clear the importance to better understand the mechanism and characterize the factors that regulate adult hippocampal neurogenesis. Furthermore, the understanding of important players in processes such as proliferation, maturation and specialization of neuronal precursors may reveal promising modulators of the adult neurogenic process with therapeutical potential. Herein, we described AP2gamma, a transcription factor known to display a role in neurodevelopmental processes, as a trigger for the generation and maturation of new glutamatergic neurons in the adult brain. Subsequently, we describe the mechanisms by which AP2gamma regulates glutamatergic neurogenesis *in vitro* (using transfected cell lines) and *in vivo* (using both AP2gamma knock-out and conditional AP2gamma mice and over-expressing retroviral vectors). Additionally, using structural, electrophysiological and behavioral end-points we demonstrated a critical role of AP2gamma in hippocampal and prefrontal cortex activity. To further clarify the importance of AP2gamma as an effective modulator of the adult hippocampal neurogenesis in a pathological context, wild-type (WT) and AP2gamma +/- mice were exposed to a chronic mild stress (CMS) protocol, which triggers depression-like features. Then, we accessed the molecular and behavioral impact of AP2gamma deletion in healthy and depressive-like animals. Results show that, under stress conditions, the heterozygous deletion of AP2gamma induces an increase of adult neurogenic progenitors consistent with a resilient effect to the CMS-induced deficits in cognitive tasks. Taken together, these evidences suggest a strong implication of AP2gamma to brain function both in physiological and pathological conditions.

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Poster

663. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant R01 MH080434

NIH Center Grant P30 HD03352

NIH Training Grant T32 GM07215

Title: Mbd1- knockout alters the progression of adult neurogenesis *in vivo*

Authors: *E. JOBE, Y. GAO, J. MLADUCKY, X. ZHAO;
Univ. of Wisconsin, Madison, WI

Abstract: MBD1 is a nuclear protein that binds to both methylated and unmethylated DNA and is thought to repress gene expression by recruiting chromatin remodeling factors. Knocking out Mbd1 in mice causes learning and memory problems that are linked to hippocampal neurogenesis as well as behavioral phenotypes that are reminiscent of Autism. These mice generate fewer mature neurons in the hippocampus, a deficit which is likely a major contributor to their behavioral phenotypes. The present study investigates the role of MBD1 in adult neural stem cells (aNSCs or NSCs) located in the dentate gyrus, a population of cells that continuously gives rise to new neurons and astrocytes in the adult brain. To study aNSCs *in vivo*, Mbd1-KO mice were crossed with mice that express GFP under the control of the nestin promoter, a protein expressed in neural stem cells. Eight week old male mice were used for these studies; females were also examined for some experiments, but no significant differences between sexes were found. Using β-gal expression, which is under the control of the endogenous Mbd1 promoter in the KO allele, we determined that MBD1 has a bimodal expression pattern throughout the stages of neurogenesis: it is expressed in stem cells and mature neurons, but not in immature neurons, or astrocytes. Contrary to expectations, Mbd1-KO mice have more nestin-GFP positive cells, as determined by stereology and FACS sorting. Yet the proportion of activated and proliferating cells within the nestin-GFP+ population is the same between KO animals and WT controls. However, there are fewer DCX+ immature neurons but more DCX /Nestin-GFP double positive cells. RNA sequencing of nestin-GFP sorted cells reveals that there are significant changes in gene expression between WT and KO animals. *In vitro*, neuronal genes are upregulated in proliferating Mbd1-KO aNSCs, yet neuronal differentiation is reduced. These results show 1) a novel expression pattern for MBD1 and 2) reveal that the loss of MBD1 alters the progression of adult neurogenesis. Understanding the role of MBD1 in aNSCs will inform our understanding how subtle alterations in the epigenetic landscape during adult neurogenesis can lead to lasting changes in the brain.

Disclosures: **E. Jobe:** None. **Y. Gao:** None. **J. Mladucky:** None. **X. Zhao:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 664.01/A106

Topic: A.02. Postnatal Neurogenesis

Support: Seid Lab, University of Scranton

Title: Neurodevelopmental effects of octopamine in isolation-induced social behaviors in Pheidole dentata

Authors: *R. S. GORE, M. A. SEID;
Biol., Univ. of Scranton, Scranton, PA

Abstract: Ants are eusocial insects that live in constant contact with their nestmates. Social behaviors like trophallaxis, allogrooming, and self-grooming function to transfer cuticular hydrocarbons onto other ants in the colony (Boulay et al. 2000a; Boulay et al. 1999, Lenoir and Hefetz, 2001; Boulay et al., 2000b). Hydrocarbon profile homogenization allows them to discriminate a nestmate from an intruder (Dahbi et al, 1999; Boulay et al. 2000a; Boulay and Lenoir, 2001). Given the importance of continuous contact with nestmates, prolonged periods of social isolation can be highly stressful and deadly (Boulay et al. 1999). Isolation increases the duration of social behaviors, and Octopamine (OA), a norepinephrine analog in invertebrates, modulates this process (Farooqui, 2012, Boulay et al. 2000a, Boulay et al. 2000b, Wada-Katsumata, 2011). The exact mechanism through which OA mediates isolation-induced social behaviors is unknown. To study the behavioral mechanism of OA on social behavior, we conducted two sets of experiments. We acutely treated adult Pheidole dentata workers with 15nL of 2.4% OA (in N,N-Dimethylformamide, DMF) following 5-day isolation, and recorded their social interactions. To determine the developmental effects of OA, we treated pupa with 15nL of 1.8% OA (in DMF) and allowed them to eclose and mature. The developed adults were then isolated for 5 days and behaviorally tested. We found an increase in duration of social behaviors, particularly allogrooming, in adults treated with OA. Surprisingly, DMF significantly lowered the duration of social behaviors compared to controls, but OA rescued these behaviors, such that OA treated ants did not significantly differ from controls or vehicle group. Therefore topical acute OA treatment had a behavior enhancing effect in adults (both isolated and non-isolated) and a protective effect during development. Detrimental effects of DMF due to chronic exposure has been well documents in mammals, however, we show that acute DMF treatment during development can be detrimental and OA may be protective against the damage.

Disclosures: R.S. Gore: None. **M.A. Seid:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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Topic: A.02. Postnatal Neurogenesis

Support: NIH AA016698

NIH 5T32AA0007462-29

W. M Keck Foundation

Title: Global and site-specific epigenetic impact of alcohol on the developing brain and partial protection by S-adenosylmethionine

Authors: *M. RESENDIZ[1], J. REITER[2], F. ZHOU[3];
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Abstract: Fetal alcohol spectrum disorder (FASD) affects approximately 1/100 children in the US. Symptoms range from severe facial dysmorphology to mild, undetectable cognitive impairment. Recent strategies such as choline and folate supplementation have shown ameliorating potential, particularly in the behavioral aspect. However, the molecular evidence informing how methionine metabolism may be involved in FASD is unclear. To assess the effects of alcohol and methionine supplementation on developing neural systems, we employed a stem cell model of neural differentiation where cells were exposed to 175 or 350mg/dL alcohol, 40mM S-adenosylmethionine (S-AMe, an active methyl donor), or all for three days. Here we report that alcohol negatively impacts DNA methyltransferase (DNMT) activity in a dose-dependent manner (2-fold decrease at 175mg/dL, $p<0.01$) and that this event is correlated with decreased DNA methylation and neural stem cell survival. Though S-AMe appeared to improve phenotypic properties, it did not significantly improve DNMT activity or global DNA methylation. Next, we extended the study to a developmental mouse model. C57 BL/6 mice were bred and introduced to an alcohol liquid diet (4% v/v), isocaloric parifed liquid diet, or alcohol liquid diet (4% v/v) supplemented with 10mM S-adenosylmethionine from embryonic days 7-16. At E17, embryonic tissues were dissected for analysis (n>4 litters/group). Examination of two DNA methylation markers abundant in the brain (5-methylcytosine, 5-meC; 5-hydroxymethylcytosine, 5-hmC) demonstrated that alcohol inhibits global DNA methylation (5-meC, cerebellum, $p< 0.05$; 5-hmC, cerebellum, $p<0.05$; 5-hmC, cortex, $p<0.05$) and that S-AMe may alleviate the negative effects of alcohol on methylation (5-meC, cerebellum, $P>0.05$; 5-hmC, cerebellum, $p<0.05$; 5-hmC, cortex, $p<0.05$). To address how alcohol-induced epigenetic inhibition may more-specifically be tied to the dysregulation of neural development, we used methylation-sensitive restriction enzyme digestion PCR and methylation-sensitive pyrosequencing to assess the effects of alcohol and S-AMe on transcriptionally-relevant sites on

the synaptic gene *Syt2* and the neural specification gene *Ngn1*. We describe that fetal exposure to alcohol decreases methylation at important CpG sites and that these can be at least partially rectified by S-AMe supplementation. This evidence supports a protective role for methionine supplementation in alcohol-related developmental outcomes and sheds greater light on the molecular basis of alcohol and epigenetics. Funding: 5T32AA0007462-29 to MR, AA016698 and W. M. Keck Foundation grant to FCZ.

Disclosures: **M. Resendiz:** None. **J. Reiter:** None. **F. Zhou:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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Topic: A.02. Postnatal Neurogenesis

Support: Fundação para a Ciência e Tecnologia (SFRH / BPD / 76642 / 2011)

IBRO International Travel Grant

Title: Role of adenosine A2A receptors on brain-derived neurotrophic factor (BDNF)-induced neurogenesis in the subventricular zone of Sprague-Dawley rats

Authors: *S. XAPELLI[1,2], F. FERREIRA[1,2], D. M. PEDRO[1,2], M. DUARTE-SAMARTINHO[1,2], F. F. RIBEIRO[1,2], A. M. SEBASTIÃO[1,2];
[1]ASebastião Lab., Inst. De Medicina Mol., Lisboa, Portugal; [2]Inst. de Farmacologia e Neurociências, Faculdade de Medicina da Univ. de Lisboa, Lisboa, Portugal

Abstract: Constitutive neurogenesis takes place in both adult mammalian subventricular zone (SVZ) and subgranular zone of the dentate gyrus. While BDNF is known to regulate SVZ neurogenesis, the role of A2A receptor (A2AR) is not known. SVZ neurospheres obtained from early postnatal (P1-3) Sprague-Dawley rats were prepared in serum-free medium (SFM) containing epidermal growth factor (EGF) and were subsequently seeded onto poly-D-lysine-coated coverslips and grown in differentiation conditions (removal of EGF). One day after plating, cells were treated with BDNF (30ng/mL), A2AR agonist CGS21680 (30nM) and/or antagonist ZM241385 (50nM). We observed that incubation with A2AR agonist or antagonist alone did not affect cell proliferation, as measured by BrdU incorporation (Control: 100.0±0.0%; CGS21680:107.8±7.2%; ZM241385: 94.3±9.5%) and neuronal differentiation, as evaluated by neuronal nuclei (NeuN) immunocytochemistry (Control: 100.0±0.0%; CGS21680:113.8±9.6%;

ZM241385: 112.8±5.2%). However, BDNF enhancement of cell proliferation (142.2±7.3%) and differentiation (162.0±10.4%) was completely prevented by A2AR antagonist (BDNF+ZM241385 (BrdU): 102.2±3.5%; BDNF+241385 (NeuN): 106.9±9.6%). For quantifying self-renewal capacity of stem/progenitor cells, SVZ dissociated cells obtained during the culture procedure were grown in SFM treated with BDNF and/or CGS21680. After 7 days, primary neurospheres were counted, collected, dissociated as single cells and grown in SFM without the drugs. After further 7 days, secondary neurospheres were counted. For the cell-fate study, dissociated SVZ cells, seeded in SFM and treated for 24 hours with the drugs, were stained for Sox2, a marker of neural stem/progenitor cells. Cell-pairs resulting from the division of a single SVZ stem/progenitor cell were counted and categorized in 3 groups according to their Sox2 expression: in both daughter cells (Sox2+/+), in only one of the daughter cell (Sox2+/-) and no expression (Sox2-/-). We showed that both A2AR activation and blockade did not change self-renewal capacity nor cell division type when comparing with control condition. Also, A2AR antagonist did not affect BDNF-mediated increase of Sox2+/+ cell-pairs. Data suggest that A2AR activation does not have a role on cell division type nor on self-renewal capacity of stem/progenitor cells. Nevertheless, besides the absence of A2ARs direct effect, the role of BDNF on cell proliferation and neuronal differentiation is dependent on the endogenous co-activation of A2ARs.

Disclosures: **S. Xapelli:** None. **F. Ferreira:** None. **D.M. Pedro:** None. **M. Duarte-Samartinho:** None. **F.F. Ribeiro:** None. **A.M. Sebastião:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 664.04/B1

Topic: A.02. Postnatal Neurogenesis

Title: Both inherent and acquired aerobic capacity protect against a chemotherapy-induced decline in cardiorespiratory and hippocampal fitness

Authors: *C. M. TOGNONI[1], N. S. NATH[1], S. M. LOOMIS[1], R. M. PEACE[2], E. A. BABB[3], E. F. O'STEEN[4], L. G. KOCH[5], S. L. BRITTON[5], L. W. JONES[6], C. L. WILLIAMS[1]; [1]Psychology & Neurosci., Duke Univ., Durham, NC; [2]Pathology, Duke Univ. Med. Ctr., Durham, NC; [3]Med., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [4]Surgery, Greenville Hlth. Syst., Greenville, SC; [5]Anesthesiol., Univ. of Michigan, Ann Arbor, MI; [6]Med., Mem. Sloan-Kettering Cancer Ctr., New York, NY

Abstract: Cardiotoxicity is one severe side effect of cancer treatments, such as anthracycline chemotherapy (doxorubicin), and can lead to congestive heart failure. Additionally, many patients experience progressive cognitive impairment following treatments for cancer − a phenomenon commonly referred to as "chemo brain." Cardiorespiratory fitness (CRF), often measured by aerobic capacity (or VO2 max) obtained from a fitness test, is thought to be one of the most powerful predictors of cardiovascular and all-cause mortality, as well as cancer survival. In rodents, voluntary wheel running has been shown to protect against the cancer treatment-induced decline in cognition and neurogenesis in the hippocampus of the adult brain, which might hint at the potential for increased CRF to be neuroprotective. However, measures of CRF are largely absent from rodent studies on the brain. To investigate whether a high level of CRF could protect against neurotoxicity as well as cardiotoxicity, four weekly rounds of doxorubicin chemotherapy were administered to rats that were selectively bred for 31 generations for low (LCR) and high (HCR) capacity for running (Koch & Britton Lab). At baseline, HCR rats have two- to three-fold higher aerobic capacity than LCR rats as measured by an incremental treadmill fitness test. We previously reported that, compared to LCR rats, HCR rats also had a two- to three-fold greater number of young neurons in the hippocampus that was correlated with highly accurate cognitive abilities. While doxorubicin induced a progressive decrease in aerobic capacity as well as neurogenesis, HCR rats remained at higher levels of aerobic capacity and neurogenesis compared to even saline-treated LCR rats. HCR and LCR rats that received individualized high-intensity interval treadmill training throughout doxorubicin treatment weeks demonstrated positive effects of exercise on both aerobic capacity and neurogenesis, regardless of inherent aerobic capacity. Overall, these findings demonstrate that inherently high CRF or increased CRF that is acquired by exercise training is protective against chemotherapy-induced damage to the heart and the brain.

Disclosures: **C.M. Tognoni:** None. **N.S. Nath:** None. **S.M. Loomis:** None. **R.M. Peace:** None. **E.A. Babb:** None. **E.F. O'Steen:** None. **L.G. Koch:** None. **S.L. Britton:** None. **L.W. Jones:** None. **C.L. Williams:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 664.05/B2

Topic: A.02. Postnatal Neurogenesis

Title: Maternal ingestion of artificial sweeteners and it's effect on the developing cerebellum

Authors: *Z. M. BAUCHI, R. A. KAREEM, A. ALHASSAN;
Ahmadu Bello Univ., Zaria, Nigeria

Abstract: Due to the proven link between consumption of sugar and tooth decay, nutritional deficiencies, diabetes, heart disease and obesity; manufacturers of many beverages and confectionaries have turned to artificial and non - nutritive sweeteners as an alternative to sugar. These sweeteners enhance the flavor of foods while reducing calories and the risk of disease. Saccharine is a non - nutritive sweetener. It is found in diet or sugar free sodas, diet coke, coke zero, desserts, sugar free gum, drink mixes, baking goods, cereal, breath mints, chewable vitamins and toothpaste. It is also one of the oldest artificial sweeteners available. Because of its health benefits and wide spread popularity, we decided to investigate the effect of maternal ingestion of saccharin on the developing cerebellum and compare it with maternal consumption of sugar. 15 pregnant female wistar rats were groups into 3, a control, sugar and saccharin group. The control group was administered distilled water, while the sugar and saccharin groups were administered with 14850mg/kg of sugar and 7100mg/kg of saccharin respectively daily throughout gestation and 2 weeks after delivery. The pups were sacrificed at birth, at 1 week and at 2 weeks of age, morphometric measurements taken and the brains harvested and processed for histological examination. Organ body weight ratio of the sugar and saccharin groups increased in comparison to the control, and the saccharin group were observed to have a greater brain weight than the sugar and control groups. Histological examinations revealed some cell damage in the saccharin group. **Keywords:** artificial sweeteners, cerebellum, development,

Disclosures: Z.M. Bauchi: None. **R.A. Kareem:** None. **A. Alhassan:** None.

Poster

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Program#/Poster#: 664.06/B3

Topic: A.02. Postnatal Neurogenesis

Support: the Hamon Center for Regenerative Science and Medicine (CRSM) Fellowship Award 2014

Title: Injury-induced neurogenesis in the adult mouse spinal cord

Authors: *L. WANG, C.-L. ZHANG;
Mol. biology, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Spinal cord injury (SCI) leads to permanent neuron loss and disruption of neural circuitry with a consequence of paralysis and long-term disability. Although neural progenitor cells exist in the adult mammalian spinal cord, they rarely produce neurons. In this study we examined whether endogenous neurogenesis can be stimulated by injuries. SCI was introduced by crush, contusion, hemi-section, or stab-wound in adult mice. Neurogenesis was analyzed by immunohistochemistry at various time points post injury. Interestingly, doublecortin (DCX)-positive neuroblasts/immature neuron-like cells were detected surrounding the lesion site at 7 dpi in all the above injury models. When compared to young (2-3 months) and middle aged (6-12 months) mice, the number of DCX-positive cells is much reduced in older (18 months) mice. Some of the DCX-positive cells found at 7 dpi are also Tuj1-positive suggesting a fate of neurons. Immunohistochemistry showed that a fraction of DCX-positive cells are proliferative. A time course analysis revealed that the number of DCX-positive cells declines rapidly with time. Ongoing experiments are aimed at examining survival and maturation of the injury-induced DCX-positive cells. Lineage mapping experiments are also conducted to determine their origin and fate. We hope to provide new insights into the injury-induced neural response and define ways for promoting intrinsic regeneration in the adult injured spinal cord.

Disclosures: L. Wang: None. **C. Zhang:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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Program#/Poster#: 664.07/B4

Topic: A.02. Postnatal Neurogenesis

Support: Lutheran Foundation

Steel Dynamics Foundation

Title: An investigation of the early cellular responses associated with an ischemic event: cortical and hippocampal profile of cellular markers EGFR, CD133, NeuN and Nestin

Authors: *G. WEMHOFF, R. SWEAZEY, B. HONG-GOKA, S. TOPALOV, F.-L. CHANG; Med. Educ., Indiana Med. Sch., Fort Wayne, IN

Abstract: Routinely, investigations centered on examining neuronal injury and recovery initiate following several hours to days after reperfusion. While these studies continue to build the large database of information regarding cellular responses, infiltrating cell populations, and potential

treatments to reduce hypoxic impact, investigations focused on examining the more immediate cellular responses to an ischemic event are limited. Central to this work is our attempting to gain insight as to why models systems that demonstrate a positive effect following exogeneous agent administration, fail to demonstrate any positive response when applied to human trials. It is established that reducing secondary responses such as immune infiltration lead to an apparent decrease in the core ischemic region. We want to look early following the ischemic event in order to eventually identify approaches that maximize the preservation and recovery of the penumbra. To this end we initiated a series of studies to examine the cellular profile/response of the cortical and hippocampal cells immediately upon the conclusion of a limited, two hour, ischemic event. Using the intraluminal suture technique, rats were exposed to two hours of ischemia without reperfusion. Animals were sacrificed and cell suspensions prepared from ischemic and nonischemic cortical and hippocampal regions and single cell suspensions were stained and subjected to flow cytometric analysis. In this study, epidermal growth factor receptor, prominin-1, the neuronal nuclear antigen and nestin were the initial targets. Early results suggest a differential expression in the percentage of EGFR and CD133 expressing cells when nonischemic naïve regions and ischemic regions were compared: EGFR naïve: cortex - 3%/hippocampus - 9%; EGFR ischemic: cortex - 12%/hippocampus - 13%, and CD133 naïve: cortex - 8%/hippocampus - 26%; CD133 ischemic: cortex - 43%/hippocampus - 33%.

Disclosures: **G. Wemhoff:** None. **R. Sweazey:** None. **B. Hong-Goka:** None. **S. Topalov:** None. **F. Chang:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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Program#/Poster#: 664.08/B5

Topic: A.02. Postnatal Neurogenesis

Support: AG034906

Title: Periodic Fasting Mimicking Diet reverses age-dependent decline in neurogenesis and enhance cognitive performance

Authors: ***I. CHOI**, P. CHILDRESS, G. NAVARRE, S. BRANDHORST, V. LONGO; USC, Los Angeles, CA

Abstract: Prolonged fasting promotes resistance to multiple stress and cause changes in aging biomarkers but its effects on longevity are poorly understood. We developed a simple diet that

mimics the effects of fasting (Fasting Mimicking Diet; FMD), and found that maintenance of the young (12 weeks old), middle age (6 months old) or the old (18 months old) mice on either a single FMD or cycles of FMD regimen resulted in a significant increase in number of proliferating cells in the dentate gyrus and the cycles of fasting mimicking diet reduces the age-dependent decline in adult neurogenesis. Furthermore, these mice also showed enhanced hippocampal dependent learning and memory performances. We demonstrated that the increase in the neurogenesis is associated with a decrease in hippocampal IGF-1 level and PKA activity as well as an increase in the neuronal differentiating and surviving factor, NeruoD1. We propose that FMD cycles can enhance cognitive performance and delay age-dependent cognitive decline in part by promoting neurogenesis.

Disclosures: I. Choi: None. **P. Childress:** None. **G. Navarre:** None. **S. Brandhorst:** None. **V. Longo:** 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.; VDL have equity interest in L-Nutra, a company that develops medical food..

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 664.09/B6

Topic: A.02. Postnatal Neurogenesis

Support: NSERC

CHIR

Title: The effects of synaptic zinc, fluoxetine and stress on adult hippocampal neurogenesis

Authors: *J. M. BOON[1], M. J. CHRUSCH[2], R. H. DYCK[1];
[1]Psychology, [2]Neurosci., Univ. of Calgary, Calgary, AB, Canada

Abstract: Adult neurogenesis has been observed in the dentate gyrus of the hippocampus and the olfactory bulbs. In adult hippocampal neurogenesis (AHN), newly formed cells migrate into the granule cell layer of the dentate gyrus where they express neuronal markers, elaborate axons, make functional synaptic connections, and improve hippocampal-dependent behaviours. The level of AHN can be modulated by a variety of factors - it is increased by exercise, environmental enrichment and exposure to selective serotonin reuptake inhibitors and decreased

by stress and ageing. We recently showed that environmental enrichment-induced increases in AHN, and improvements in hippocampal-dependent behavioural tasks were ablated in animals lacking synaptic zinc (ZnT3-knockout (KO) mice). Here, we examine whether synaptic zinc is also essential for modulation of AHN by other factors, specifically, exposure to fluoxetine and/or to stress. Female ZnT3-WT and -KO mice were chronically exposed to fluoxetine or vehicle, while in stressed (single-housed) or non-stressed (pair-housed) conditions for 6 weeks. All animals then underwent behavioural testing (elevated plus maze, novelty-suppressed feeding, pattern separation), and levels of AHN were assessed. We found that synaptic zinc is necessary for fluoxetine-induced increases in AHN, in both stressed and non-stressed mice. We also found that behavioural benefits associated with fluoxetine-induced increases in AHN are ablated in all stressed animals. In non-stressed animals, only WT animals demonstrate the behavioural benefits of fluoxetine thus indicating that synaptic zinc is necessary for the behavioural benefits of fluoxetine. These data implicate synaptic zinc as an essential modulator of AHN, acting upstream of the effects of environmental enrichment and fluoxetine.

Disclosures: J.M. Boon: None. **M.J. Chrusch:** None. **R.H. Dyck:** None.

Poster

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Topic: A.02. Postnatal Neurogenesis

Support: NIH/NIGMS COBRE: The Delaware Center for Neuroscience Research Grant 1P20GM103653 - 01A1

Title: Long-term impact of neonatal ethanol exposure on hippocampal adult neurogenesis, BDNF expression, and bdnf DNA methylation in rats

Authors: *K. E. BOSCHEN[1], K. J. CRISS[2], T. L. ROTH[1], A. Y. KLINTSOVA[1];
[1]Psychology, Univ. of Delaware, Newark, DE; [2]Psychological and Brain Sci., Univ. of Delaware, NEWARK, DE

Abstract: Exposure to ethanol *in utero* in humans may result in neuroanatomical, cognitive, behavioral, and physiological deficits. Certain brain structures, including the hippocampus, are particularly sensitive to teratogenic insult during the “brain growth spurt” (occurring during the third trimester in humans and first two postnatal weeks in rodents), potentially contributing to the memory and behavioral deficits observed in models of developmental ethanol exposure. Ethanol

exposure during the third trimester-equivalent has been shown to negatively impact hippocampal neuroplasticity, increase apoptosis, decrease dendritic morphology, and impair adult neurogenesis and LTP. The current study investigates the effects of a binge ethanol exposure during neonatal development on expression of brain-derived neurotrophic factor (BDNF) and exon-specific BDNF mRNA transcripts in adulthood. On postnatal days (PD) 4-9, ethanol-exposed rat pups (AE) were intragastrically intubated 2x daily with 5.25 g/kg/day ethanol. Sham-intubated (SI) animals were intubated alongside AE pups without liquid and suckle control (SC) animals were undisturbed. Animals were assigned to one of three housing conditions from PD30-72: social housing (SH), wheel running only (WRWR), or twelve days of WR followed by living in a complex environment (WREC), and sacrificed on PD72. Current data suggests that ethanol exposure does not have a long-term effect on new cell proliferation at PD72 (as measured by Ki-67 and doublecortin immunocytochemistry), but impacts long-term cell survival and dendritic complexity of dentate gyrus granule cells, resulting in less total dendritic material. Basal BDNF mRNA and protein expression does not seem to be affected by ethanol exposure at PD72, suggesting that maintaining changes to dendritic complexity occurs through other mechanisms. A differential effect of WRWR and WREC on BDNF gene expression was found, with WRWR increasing gene expression while WREC did not. Previous work from our lab shows that ethanol exposure affects BDNF expression and DNA methylation shortly following the ethanol exposure (PD10), however the current data suggest that these alterations become more subtle across the lifespan. Impairments to adult neurogenesis suggest that ethanol-induced alterations to expression of other plasticity-related factors may play a role as well. Supported by NIH/NIGMS COBRE: The Delaware Center for Neuroscience Research grant 1P20GM103653 - 01A1 to AYK.

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Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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Topic: A.02. Postnatal Neurogenesis

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

VA Merit Award to A.K.S.

Title: Voluntary exercise mediated enhanced neurogenesis does not impair the recall of retrograde memory

Authors: *A. K. SHETTY[1,2], M. KODALI[1,2], T. MEGAHED[1], V. MISHRA[1], B. SHUAI[1,2], X. RAO[1,2], B. HATTIANGADY[1,2];
[1]Mol. and Cell. Med., Inst. for Regenerative Med, TAMHSC Col. of Med., Temple, TX; [2]Res. Service, Olin E. Teague Veterans' Med. Center, Central Texas Veterans Hlth. Care Syst., Temple, TX

Abstract: Physical exercise is beneficial for boosting cognitive and mood function as well as for easing neuropsychiatric disorders. Many studies have implied that enhanced neurogenesis in the hippocampus is one of the substrates mediating these favorable effects. Yet, a recent study using mouse model has shown that running-induced increased neurogenesis impairs the recall of hippocampus-dependent memories that were made prior to the exercise regimen (Akers et al., Science 344: 598-602, 2014). The study also proposed that reconfiguration of the DG-CA3 circuitry through addition of greater numbers of granule cells reduces the ability of a given set of cues to re-invoke the same pattern of activity that occurred at the time of memory formation as a possible reason for the degradation of retrograde memories. This interesting finding raised a vital question whether such forgetting induced by running (via enhanced neurogenesis) is applicable to all mammals. Hence, we ascertained whether voluntary running exercise would promote forgetting in young male F344 rats. Animals were first subjected to 8 sessions of water maze training followed by a probe test (PT) at 24 hrs after the last training session. Animals displaying similar memory recall ability were next assigned randomly to two groups: an exercise group in which rats were individually housed in larger cages with access to running wheels (n=14) and a sedentary group where rats were housed in standard cages (n=15). Animals also received 5'-bromodeoxyuridine injections on days 15-17 of the running/sedantary period. Animals in the exercise group ran an average of 1.2 Kms/day and 31 kms for the entire 28-day duration. A second PT conducted after 28-day housing as above revealed similar ability for memory recall between the exercise and sedentary groups, evidenced through increased dwell times in the platform quadrant vis-à-vis other quadrants. Comparison of additional parameters of memory recall revealed the same. Analyses of the DG in animals euthanized a week after the 2nd PT using doublecortin immunostaining revealed that neurogenesis was increased by ~1.5 folds in the exercise group. A similar increase was seen when net neurogenesis was quantified in animals euthanized 4 weeks after the 2nd PT. These results are in sharp contrast to findings in the previous mouse study. The discrepancy likely reflects differences in kilometers ran, the extent of exercise-induced increase in neurogenesis and the type of memory test employed. Nonetheless, our results provide novel evidence that moderate physical exercise induced increases in neurogenesis do not interefere with the recall of memory that was formed prior to the exercise regimen.

Disclosures: A.K. Shetty: None. **M. Kodali:** None. **T. Megahed:** None. **V. Mishra:** None. **B. Shuai:** None. **X. Rao:** None. **B. Hattiangady:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 664.12/B9

Topic: A.02. Postnatal Neurogenesis

Support: VA Merit Award to A.K.S.

Title: Curcumin improves memory and mood with enhanced neurogenesis and alleviation of inflammation and oxidative stress in a model of gulf war illness

Authors: *M. KODALI[1,2], B. HATTIANGADY[1,2], G. SHETTY[1,2], B. SHUAI[1,2], X. RAO[1,2], A. K. SHETTY[1,2];
[1]Inst. for Regenerative Med, TAMHSC Col. of Med., Temple, TX; [2]Res. Service, Olin E. Teague Veterans' Med. Center, Central Texas Veterans Hlth. Care Syst., Temple, TX

Abstract: Brain dysfunction in Gulf War Illness (GWI) mainly includes memory and mood impairments. Based on epidemiological studies, exposures to anti nerve gas agent pyridostigmine bromide (PB), pesticides and stress during the war have caused GWI in a major fraction of Persian Gulf War-1 veterans. Indeed, our studies in a rat model have shown that exposures to GWI-related chemicals (GWIR-Cs) and mild stress for 4 weeks cause persistent memory and mood dysfunction linked with declined neurogenesis, increased oxidative stress, and low-grade inflammation in the hippocampus. Hence, drugs and compounds capable of improving memory and mood function through increases in neurogenesis and/or suppression of oxidative stress and inflammation are of great interest for treating GWI. Curcumin (CUR), a polyphenol derived from the rhizome of curcuma longa, has neurogenic and antiinflammatory properties. Administration of CUR is also considered safe in humans and has minimal side effects. Therefore, we tested the usefulness of curcumin therapy in a rat model of GWI. Male SD rats were exposed daily to GWIR-Cs, DEET (40 mg/kg), permethrin (0.13 mg/kg) and PB (1.3 mg/kg) and 5-minutes of restraint stress for 4 weeks. A week later, a group of rats received daily CUR (30 mg/kg, i.p) for 4 weeks while another group received vehicle (VEH). Animals also received 5'-bromodeoxyuridine (BrdU) during the last 7 days of treatment for analysis of neurogenesis. Behavioral tests performed 4 weeks later revealed improved memory function in CUR treated GWI-rats, which was evidenced through their preference to explore an object that was displaced to a novel location in object location test or to explore a novel object over a familiar object in novel object recognition test. Performance in a novelty suppressed feeding test implied improved mood function as well. These rats also displayed three-fold increase in hippocampus neurogenesis (measured via BrdU, BrdU-NeuN, and doublecortin+ cell counts). Moreover, CUR-treated GWI-rats exhibited reduced inflammation, typified by diminished astrocyte

hypertrophy and ED-1+ activated microglia in the hippocampus. Besides, the hippocampus of these animals displayed diminished concentration of oxidative stress markers 4-HNE and 3-NT, and increased expression of antioxidant genes. Thus, CUR therapy is efficient for alleviating memory and mood dysfunction in GWI. In view of the role of neurogenesis, inflammation and oxidative stress in altering memory and mood function, it is likely that CUR-induced enhanced neurogenesis, and repressed inflammation and oxidative stress triggered the beneficial effects.

Disclosures: **M. Kodali:** None. **B. Hattiangady:** None. **G. Shetty:** None. **B. Shuai:** None. **X. Rao:** None. **A.K. Shetty:** None.

Poster

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the Ichiro Kanehara Foundation

Title: The 5-HT3 receptor is essential for exercise-induced hippocampal neurogenesis and antidepressant effects

Authors: M. KONDO, Y. NAKAMURA, Y. ISHIDA, *S. SHIMADA;
Grad. Sch. of Medicine, Osaka Univ., Osaka, Japan

Abstract: Exercise has a variety of effects on the animal brain at many levels. At the cellular level, exercise enhances hippocampal neurogenesis. At the behavioral level, exercise induces antidepressant effects and improves learning behavior. It has been suggested that neurotransmitters and neurotrophic factors, such as serotonin (5-hydroxytryptamine, 5-HT),

BDNF (brain-derived neurotrophic factor), IGF-1 (insulin like growth factor-1) and VEGF (vascular endothelial-derived growth factor), are candidate factors that mediate these exercise effects on the brain. 5-HT has attracted much attention in the context of the functional link between major depression and adult neurogenesis in the hippocampal dentate gyrus. Actually, the dentate gyrus is highly enriched with serotonergic fibers, and the 5-HT system regulates neuronal plasticity in the dentate gyrus. Additionally, the beneficial effects of antidepressant drugs that primarily target the central 5-HT system have been shown to require the generation of new hippocampal granule neurons. Previous studies have shown that both 5-HT and exercise have neurogenic and antidepressant effcts, and have significant effects on learning and memory. Furthermore, exercise increases the levels of 5-HT in the hippocampus, which could affect learning and affective processes. However, the precise mechanism of action of 5-HT in neurogenic and behavioral effects induced by exercise is unknown. The 5-HT3 receptor is the only ionotropic receptor in the 5-HT receptor subfamilies. The 5-HT3 receptor is expressed on interneurons in the limbic region, including the hippocampus, amygdala and prefrontal cortex. However, its possible role in hippocampal neurogenesis has not been defined. Previous studies have indicated that the 5-HT3 receptor plays important roles in mood and memory. Thus, we have investigated the possible relationship of the 5-HT3 receptor with exercise-induced hippocampal neurogenesis and behavioral changes. In this study, analysis of the 5-HT3A receptor subunit-deficient (htr3a-/-) mice revealed that lack of the 5-HT3 receptor resulted in loss of exercise-induced hippocampal neurogenesis and antidepressant effects, but not of learning enhancement. Furthermore, stimulation of the 5-HT3 receptor promoted neurogenesis. These findings demonstrate that the 5-HT3 receptor is the critical target of 5-HT action in the brain following exercise, and is indispensable for hippocampal neurogenesis and antidepressant effects induced by exercise. This is the first report of a pivotal 5-HT receptor subtype that plays a fundamental role in exercise-induced morphological changes and psychological effects.

Disclosures: **M. Kondo:** None. **Y. Nakamura:** None. **Y. Ishida:** None. **S. Shimada:** None.

Poster

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Topic: A.02. Postnatal Neurogenesis

Support: FONDECYT Grant 1140477

CMA BIO BIO CONICYT Grant ECM-12

Title: Vitamin C deficiency disrupts normal cellular composition within the subventricular zone neurogenic niche

Authors: *N. A. JARA[1], M. CIFUENTES[2], F. MARTÍNEZ[1], K. SALAZAR[1], F. NUALART[1]; [1]Univ. De Concepción, Concepción, Chile; [2]Univ. de Málaga, Málaga, Spain

Abstract: The subventricular zone (SVZ) is the largest neurogenic niche in the adult brain. It is mainly formed by: SVZ astrocytes (B cells), which correspond to the adult neural stem cells; neuroblasts (A cells); type C cells; and ependymal cells (E cells). In the guinea pig brain, neuroblasts born in the SVZ migrate to the olfactory bulb (OB), where they surround a continuous open ventricle known as the lateral ventricle extension (LVE), which reaches the OB. Previous studies suggest that vitamin C could modulate neurogenesis. In the embryonic brain, vitamin C induces the differentiation of precursor cells into neurons and astrocytes. In the adult brain, guinea pigs subjected to vitamin C deficiency show a lower number of hippocampal neurons. However, no studies have assessed the effect of a vitamin C deficiency on the adult SVZ. Therefore, we analyzed the effect of this condition in the SVZ and LVE of the adult guinea pig brain. Guinea pigs consumed a vitamin C-deficient diet for 0, 2, and 3 weeks. Cell proliferation was analyzed using BrdU-labeling. Immunohistochemical analysis, confocal spectral microscopy, and transmission electron microscopy were used to compare the different cell populations within the SVZ and LVE of control and vitamin C-deficient animals. We identified neuroblasts (βIII-tubulin+), SVZ astrocytes (vimentin+), type C cells (BrdU+), and ependymal cells (vimentin+ and isolectin B4+) in the SVZ and LVE of control and vitamin C-deficient animals. Neuroblasts were reduced as the days of deficiency increased. We demonstrated a significant and progressive reduction that reached about 50% in the SVZ and LVE with 21 days of deficiency. This reduction in neuroblasts was correlated with the reduction of BrdU+ cells in the SVZ and LVE. In the ultrastructure analysis, the SVZ looked similar in deficient and control animals; however, the deficient animals had fewer neuroblasts and, therefore, the subependymal cells were mainly astrocytes. In addition, the ultrastructure of the LVE revealed that the cellular and nuclear morphology of the ependymal cells was different in deficient versus control animals and the former had fewer neuroblasts; however, the same or even more astrocytes were observed. We showed that vitamin C is essential for the maintenance of the SVZ cell populations required for normal activity of the SVZ neurogenic niche in the adult guinea pig brain. Moreover, we observed similar results in the LVE, suggesting that a vitamin C deficiency may modify the normal OB structure and neuronal composition. Finally, vitamin C may be modulating the neurogenic activity and potential of the SVZ neurogenic niche.

Disclosures: **N.A. Jara:** None. **M. Cifuentes:** None. **F. Martínez:** None. **K. Salazar:** None. **F. Nualart:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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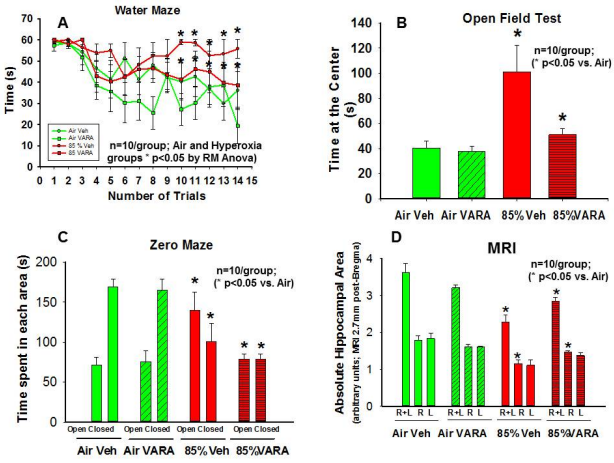
Program#/Poster#: 664.15/B12

Topic: A.02. Postnatal Neurogenesis

Title: Retinoids attenuate neonatal hyperoxia-induced neurodevelopmental impairment in mice

Authors: ***M. RAMANI**[1], N. AMBALAVANAN[2], T. VAN GROEN[3], I. KADISHA[3]; [1]Pediatrics, Univ. of Alabama At Birmingham, Birmingham, AL; [2]Pediatrics, [3]Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Background: Extremely preterm infants exposed to supraphysiological oxygen exposure during a critical developmental period often have poor executive and memory function during later life associated with reductions in hippocampal volume. We recently showed that adult mice exposed to neonatal hyperoxia had deficits in spatial and recognition memory associated with smaller hippocampal volumes. Retinoids have been shown to attenuate hyperoxia induced lung injury in animal models and reduce neonatal chronic lung disease in human preterm infants. We hypothesized that retinoid administration during neonatal hyperoxia exposure would also inhibit hyperoxia-induced brain injury. Methods: C57BL/6 mouse pups (n = 2 litters per condition; at least 10/group) were exposed to hyperoxia (85% oxygen) or air, in combination with either Vitamin A+ Retinoic Acid (VARA) or canola oil (vehicle) from postnatal day 2 to 14 (P2-P14; roughly corresponding from early preterm to early infancy in human brain development) and then returned to air. Neurobehavioral and structural assessments were done at 12-14 weeks of age. Results: Neonatal hyperoxia induced spatial navigation deficits and increased exploratory behavior in adult mice, and these changes were attenuated by the administration of VARA (Figure A, B, C). Neonatal hyperoxia also reduced adult right hippocampal size, and this effect was also attenuated by VARA administration (Figure D). Brain sections stained for white matter (CNPase), astrocytes (GFAP) and synaptic density (synaptophysin) were qualitatively similar among the groups. Conclusion: Administration of VARA during neonatal hyperoxia attenuates adult neurodevelopmental deficits and associated reduction in hippocampal size in mice. This animal model suggests retinoids may be a neuroprotective agent in extremely preterm infants at high risk of neurodevelopmental impairment.



Disclosures: **M. Ramani:** None. **N. Ambalavanan:** None. **T. van Groen:** None. **I. Kadisha:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 664.16/B13

Topic: A.02. Postnatal Neurogenesis

Support: BSGPE

FNRS J.0065.15

NS 080979

Ellison Medical Foundation

Title: Effects of perinatal exposure to PCBs on thyroid hormones, spinogenesis and neurogenesis in mice dentate gyrus

Authors: ***A. PINSON**[1], A. S. PARENT[1], N. WOODS[2], C. CHRISTINA[2], A. BENSEN[2], A. GERARD[1], E. NAVEAU[1], J. P. THOME[3], J. P. BOURGUIGNON[1], G. L. WESTBROOK[2]; [1]unit of neuroendocrinology, Univ. of Liege, Giga Neurosciences, Liege, Belgium; [2]Vollum Institute, Oregon Hlth. and Sci. Univ., Portland, OR; [3]Lab. of Animal Ecology and Ecotoxicology (LEAE, CART), Univ. of Liège, liege, Belgium

Abstract: Perinatal exposure to polychlorinated biphenyls (PCBs), an endocrine disruptor, is associated with relative hypothyroidism and learning and memory deficits in children and rodents. We hypothesized that perinatal exposure to Aroclor 1254 (A1254), a mixture of PCBs, from gestational day 6 (E6) to postnatal day 21 (P21) could impair neurogenesis in the dentate gyrus in C57BL/6J WT and proopiomelanocortin-enhanced green protein (POMC-EGFP) mice, the latter of which transiently and selectively express EGFP in newborn neurons in the dentate gyrus. Total thyroxin concentration was decreased in serum of A1254-exposed mice at P7 and P21 but not at P56. Despite the decreased thyroxin levels, early exposure did not affect the proliferation or survival of newborn neurons at P7 as assessed using POMC-EGFP mice as well as BrdU pulse labeling. However, 5 weeks after the end of the exposure (P56), the number of POMC-EGFP+ cells was moderately but significantly reduced in the dentate gyrus of exposed mice (105±8.41 vs 81±3.58 cells/section; control vs exposed). This effect was compensated by 2 weeks of voluntary running (138.6±17.07 vs 142±7.29 cells/section; control vs exposed). In mice exposed to PCBs, dendritic spine density of 2 weeks post-mitosis neurons labeled by a stereotaxic injection of a retrovirus expressing EGFP was transiently increased on P21 (1.17±0.09 spines/µm vs. 0.86±0.07; exposed vs control). This effect was not observed in mature neurons labeled by Golgi staining at P21. Exposure to A1254 prevented the normal increase in frequency of spontaneous excitatory postsynaptic currents (sEPSC) in newborn neurons between14 and 21 days post-mitosis. The mean amplitude of sEPSC was not affected by A1254 exposure but A1254 significantly increased the variability of sEPSC amplitudes, which may suggest a disruption of normal synaptic maturation. Our results suggest that early exposure to PCBs can alter excitatory synapse development during a period of active synaptogenesis.

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Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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Topic: A.02. Postnatal Neurogenesis

Support: Consejería de Innovación, Ciencia y Empleo, Junta de Andalucía, España (grant numbers P10CTS6639 and P07- FQM-02925).

Title: 12-deoxyphorbols isolated from Euphorbia resinifera promote neural stem cell proliferation and adult neurogenesis via PKC activation

Authors: N. GERIBALDI-DOLDÁN[1], E. FLORES-GIUBI[2], M. MURILLO-CARRETERO[1], F. GARCÍA-BERNAL[1], M. CARRASCO[1], A. J. MACÍAS-SÁNCHEZ[2], J. DOMÍNGUEZ-RISCART[1], C. VERÁSTEGUI[1], R. HERNÁNDEZ-GALÁN[2], *C. CASTRO[1];
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Abstract: Generation of new neurons from neural stem cells is induced in the central nervous system in response to different types of injuries. Strategies aimed to facilitate neuronal renewal by promoting neurogenesis constitute a promising therapeutic option to treat brain damage associated with neuronal death. It is well known that cell proliferation is increased by Protein Kinase C (PKC) activated mechanism. In this contex, we have found that PKC activation by phorbol 12-myristate 13-acetate promotes neural progenitor cell (NPC) proliferation. In addition, the capacity of the non-tumorigenic PKC activator prostratin, as well as other 12-deoxyphorbols isolated from *Euphorbia resinifera* sharing structural similarities with prostratin were tested for their capacity to affect NPC proliferation. Our results showed that prostratin promoted NPC proliferation and this effect was reverted by the general PKC inhibitor G06850. Additionally, we analyzed the molecular mechanisms involved showing that prostratin activated the expression of cyclin D, E and A. Similar proliferative effects were obtained with all the 12-deoxyphorbols tested, which induced NPC proliferation in a PKC-dependent fashion. Most of the 12-deoxyphorbols evaluated were significantly more potent than prostratin in inducing NPC proliferation. Additionally, we show that treatment with several 12-deoxyphorbols *in vivo* induces proliferation of NPCs within the main neurogenic areas of the adult brain: the dentate gyrus of the hippocampus and the subventricular zone. We suggest that PKC activation might be a promising strategy to expand the endogenous NPC population to promote neurogenesis. Our results support the potential of 12-deoxyphorbols as new pharmaceutical agents to facilitate neuronal renewal. This work was funded by the Consejería de Innovación, Ciencia y Empleo, Junta de Andalucía, España (grant numbers P10CTS6639 and P07- FQM-02925), and by Fundación Rodrçiguez Pascual 2015.

Disclosures: N. Geribaldi-Doldán: None. **E. Flores-Giubi:** None. **M. Murillo-Carretero:** None. **F. García-Bernal:** None. **M. Carrasco:** None. **A.J. Macías-Sánchez:** None. **J. Domínguez-Riscart:** None. **C. Verástegui:** None. **R. Hernández-Galán:** None. **C. Castro:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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Program#/Poster#: 664.18/B15

Topic: A.02. Postnatal Neurogenesis

Support: Nipissing University

Title: Social isolation, ethanol, and golgi in planarians

Authors: B. LOVELL, T. MCCHARLES, N. LANDRY, *A. D. STILLAR, A. WEEKS, M. SAARI;
Nipissing Univ., North Bay, ON, Canada

Abstract: We have recently developed a method of Golgi staining of planarians (Dugesia dorotocephala) based on a combination of methods used in mammalian brains. This method allows for observation of neural plasticity associated with environmental toxins or manipulation of the social environment in planaria. In order to examine the potential toxic effects of ethanol (EtOH) at low dosages and the effect of social isolation, planarians were placed into isolation, exposed to EtOH, and neuron counts as well as other anatomical measures were taken. Isolated or control (housed in groups of ten) planarians were placed in one of four conditions of EtOH: vehicle (30ml dechlorinated H2O), 1.0%, 1.5%, or 2.0% for five minutes and then stained. The EtOH concentrations were chosen based on a pilot study to confirm EtOH thresholds that cause complete immobility of the planarians. MANOVA of the 2 X 4 design revealed significant EtOH effects on the length of the anterior to posterior axis, eye spot to tip and a few other effects. The neural count approached significance as a function of social condition. Although the multivariate interaction was not significant (p. = .175) examination of the associated univariate F values suggested that some measures may be sensitive to the combined effects of EtOH and the social manipulation. (Supported by Nipissing University).

Disclosures: B. Lovell: None. **T. Mccharles:** None. **N. Landry:** None. **A.D. Stillar:** None. **A. Weeks:** None. **M. Saari:** None.

Poster

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Topic: A.02. Postnatal Neurogenesis

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

VA Merit Award to A.K.S.

Title: Early alterations in the mouse brain following an exposure to shock waves of a single blast

Authors: *D. UPADHYA[1,2], A. B. ROBBINS[3], B. HATTIANGADY[1,2], M. KODALI[1,2], B. SHUAI[1,2], G. A. SHETTY[1,2], M. MORENO[3], A. K. SHETTY[1,2];
[1]Insti. Regenerative Med, TAMHSC Col. of Med., Temple, TX; [2]Res. Service, Olin E. Teague Veterans' Med. Center, Central Texas Veterans Hlth. Care Syst., Temple, TX; [3]Dept of Biomed. and Mechanical Engin., Texas A&M Univ., College Station, TX

Abstract: Exposure to blast shock waves (BSWs) causes mild traumatic brain injury (mTBI) and diverse neurological deficits varying on the severity of shock waves (SWs) and the site of brain affected by SWs. Exposure to BSWs underlies a significant fraction of combat related mTBI incurred by Operation Iraqi Freedom and Operation Enduring Freedom veterans. Although brain imaging studies do not typically reveal sites of brain injury at early post-exposure time points, blast-related mTBI can lead to memory and mood dysfunction several years after the incident. Indeed, our previous study using a mouse model has shown that exposure to shock waves of a single blast can cause impairments in memory, mood and hippocampus neurogenesis, when examined six months after the exposure. Here, we examined early changes in the mouse brain after an exposure to SWs of a single blast. We wrapped each anesthetized C57BL/6 mouse in a flexible Kevlar and placed inside a Schedule 80 PVC container designed to leave only the head exposed. The container was then fixed to the distal end of the shock tube apparatus, and the head was exposed once to BSWs (12 psi) via restrained rupture of a Mylar membrane. Age-matched mice served as sham controls, which were anesthetized and placed near the shock tube to receive only the sound. Analyses of brains at 24 hrs and 14 days post-exposure suggested increased activity of GFAP+ astrocytes, IBA-1+ microglia and/or NG2+ oligodendrocyte progenitors in the cerebral cortex, corpus callosum and internal capsule. The hippocampus did not show noticeable changes in GFAP+ or IBA-1+ elements in most areas but microscopic patches of injury and inflammatory reaction could be observed in the CA1 region, where long IBA-1+ microglial processes were concentrated at high density. Characterization of the subgranular zone (SGZ) of the hippocampus with Ki-67 revealed increased proliferation of cells at both 24 hrs and 14 days after exposure to BSWs. Quantification of putative neural stem cells (NSCs) in the SGZ via Sox-2 and Ki-67 dual immunofluorescence did not show differences in proliferation between sham- and BSW-exposed animals, suggesting that cells other than NSCs may be proliferating in the SGZ after exposure to BSWs. Moreover, stereological measurement of newly born neurons positive for doublecortin at 14 days post-exposure revealed no differences in the overall status of neurogenesis between sham- and BSW-exposed animals. Thus, exposure of the brain to SWs of a single blast causes modest changes in astrocytes, microglia and oligodendrocyte progenitor cells in several brain regions but does not alter the extent of hippocampus neurogenesis at early time-points after the exposure.

Disclosures: **D. Upadhya:** None. **A.B. Robbins:** None. **B. Hattiangady:** None. **M. Kodali:** None. **B. Shuai:** None. **G.A. Shetty:** None. **M. Moreno:** None. **A.K. Shetty:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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Topic: A.02. Postnatal Neurogenesis

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PTDC/SAU-FCF/098685/2008 Project, Foundation for Science and Technology (FCT), Portugal

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Grant from the Institut du Cerveau et de la Moelle épinière, Paris

Title: Methamphetamine promotes neuronal differentiation and strengthens long-term potentiation of immature dentate granule neurons

Authors: *S. BAPTISTA[1], F. BORGES[2], N. MILHAZES[2], A. SILVA[3], A. BACCI[4]; [1]Lab. of Pharmacol. and Exptl. Therapeutics, IBILI, Fuculty of Medi, Coimbra, Portugal; [2]Fac. of Sciences, Univ. of Porto, 3CIQUP/Department of chemistry and Biochem., Porto, Portugal; [3]Lab. of Pharmacol. and Exptl. Therapeutics, IBILI, Fuculty of Medicine, Univ. of Coimbra, Coimbra, Portugal; [4]Sorbonne Universite´s UPMC Univ. Paris 06, UMR S 1127, Paris, France, 7Inserm U 1127, Paris, France, 8CNRS UMR 7225, Paris, France

Abstract: Methamphetamine (METH) is a psychostimulant drug of abuse, whose consumption has been increasing worldwide. Several studies report that METH causes irreversible brain abnormalities that may reflect cognitive deficits. Despite the fact that the underlying mechanisms are still not well characterized, it has been suggested that alterations of dentate gyrus (DG) neurogenesis may contribute to these deficits. Accordingly, several pieces of evidence have suggested that METH impairs hippocampal neurogenesis, which can in part justify memory

deficits observed in METH abusers. However, little is known about the effect of METH on synaptic plasticity of DG neurons. Here, we aimed at investigating the impact of METH on neurogenesis and synaptic plasticity of immature and mature dentate granule cells. We used the GAD67-GFP (line G42) mice, in which GFP is transiently expressed in maturating newborn neurons. Male G42 mice were administered with 2 mg/kg/day METH (i.p.) once a day for 7 days. 24 h after the last METH injection, immature neuronal phenotype, synaptic plasticity, dendritic and spine morphology were assessed. We found that METH treatment enhanced the differentiation of immature GFP+ cells shifting from DCX- to a more mature NeuN-expressing GFP+ cells. Electrophysiologically, GFP+ neurons showed three different profiles, reflecting specific stages of differentiation. We found that METH treatment had little effect on long-term potentiation (LTP) of synaptic transmission onto very young neurons but facilitated and strengthened LTP in more differentiated GFP+ neurons and mature GFP- DG neurons, respectively. Interestingly, despite METH did not induce any change dendritic morphology, it differently altered spine morphology in GFP+ DG cells at specific maturation stages. In conclusion, METH interferes with DG neurogenesis by accelerating differentiation of immature neurons and tends to facilitate LTP more differentiated GFP+ and in mature GFP- DG neurons. These results might have important implications towards the understanding of the synaptic basis of METH-induced cognitive deficits.

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Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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Topic: A.02. Postnatal Neurogenesis

Support: CTSA Translational Science Training Grant TL1 TR001119

Nathan Shock Biology of Aging Training Grant T32 AG021890

Nathan Shock Center of Excellence in Basic Biology of Aging-Pilot Grant

Title: Calorie restriction protects against age-related dysregulation of neural stem cells in the murine subventricular zone

Authors: *D. M. APPLE[1], R. SOLANO FONSECA[1], M. C. TEXIERA DOS SANTOS[2], S. MAHESULA[2], C. ZHU[1], E. KOKOVAY[2];
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Abstract: Aging is a major risk factor for increased susceptibility to damage from brain insults like stroke, inflammation, and disease. Calorie restriction (CR) can improve physiological markers of health during aging, including extending lifespan and protecting against age-related damage to the brain. The largest source of neural stem cells in the adult brain is the subventricular zone (SVZ). We sought to determine the effect of long-term CR on neurogenesis and the neural stem cell niche in the SVZ of young and aged mice. Here, we show that aged mice fed standard control chow have fewer SVZ-derived neurons in the olfactory bulb, indicating that aging impairs neural stem cell function. Long-term CR preserved neural stem cell function and resulted in a significant increase in neurogenesis in aged mice compared with ad libitum- fed controls. Paradoxically, we have observed that proliferation of neural stem cells is decreased in aged CR mice. This is in the presence of increased neuroblast formation and increased neurogenesis. These data indicate either a change in cell fate or a change in the survival of transit amplifying cells as they mature into neuroblasts, suggesting an altered regulatory mechanism. Confocal imaging and fluorescent staining of SVZ wholemounts revealed an increase in both the total number and reactivity of microglia in the aged control mouse, suggesting increased inflammation in the neural stem cell niche during aging. Remarkably, these age-related inflammatory markers were not observed in the long-term CR aged mice, which appeared no different from young control and young CR mice included in the study. We have found that the neural stem cell chemoattractant mechanism CXCL12, secreted by endothelial cells, and its receptor expressed on neural stem cells, CXCR4, are dysregulated in the aged mouse fed ad libitum, but not in the aged CR mouse. Further, the recently identified rejuvenation factor GDF11 was found to be decreased in the aged SVZ, but not in the aged CR SVZ. These initial experiments have revealed a protective role for CR in the aging SVZ, and are an important first step in understanding how CR may be an effective therapeutic intervention for the aging or damaged brain.

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Poster

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Support: VA Merit Award to A.K.S.

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

Title: Subventricular zone NSC grafting into the hippocampus after status epilepticus modifies disease by curtailing epileptogenesis and inflammation

Authors: *G. ZANIRATI[1,2,3], B. HATTIANGADY[1,2,3], B. SHUAI[1,2,3], J. BLAIR[1,2], A. K. SHETTY[1,2,3];
[1]Inst. for Regenerative Med., Texas A&M Hlth. Sci. Ctr. Col. of Med., Temple, TX; [2]Inst. for Regenerative Med., Temple, TX; [3]Olin E. Teague Veterans' Med. Center, Central Texas Veterans Hlth. Care Syst., Temple, TX

Abstract: Status epilepticus (SE) evolves into chronic temporal lobe epilepsy (TLE) through epileptogenic changes and persistent inflammation in the hippocampus. The features of TLE include complex partial seizures and impaired memory and mood function. Several evolving epileptogenic changes are believed to contribute to these impairments, which include aberrant and reduced neurogenesis, abnormal mossy fiber sprouting, and diminished numbers of inhibitory interneurons. Persistent inflammation is another alteration contributing to the disease. Subventricular zone neural stem cells (SVZ-NSCs) have shown ability to promote brain repair in several neurological disease prototypes with neuroprotective and antiinflammatory effects. Hence, using an animal model of SE, we rigorously examined whether grafting of SVZ-NSCs early after SE would provide lasting control over spontaneous recurrent seizures (SRS), and whether such alleviation of SRS would be linked with curbs on epileptogenesis and inflammation. We induced SE in young male rats via graded intraperitoneal injections of kainic acid and terminated acute seizures two hrs after SE induction by diazepam injection. Four days after SE, a group of animals received SVZ-NSC grafts into the hippocampus (4 grafts/side, ~90,000 live cells/site). Another group was maintained as epilepsy-only controls. Analyses at 6-months after SE using video-electroencephalographic recordings revealed that animals receiving NSC grafts displayed reduced frequency and intensity of SRS, in comparison to epilepsy-only controls. The reductions were ~60% for the frequency of all SRS, ~97% for Stage-V SRS and ~72% for the percentage of time spent in seizure activity. Grafted animals also displayed preserved object recognition memory function and reduced depressive-like behavior. Analyses of the hippocampus in these animals revealed persistence of NSC-graft derived cells (equivalent to ~89% of injected cells) and their differentiation into GABA-ergic interneurons (~20%) and astrocytes (~60%). Moreover, NSC grafting promoted lasting restraint on several epileptogenic changes and inflammation in the hippocampus. In contrast to epilepsy-only controls, the hippocampus of grafted animals displayed normal pattern and greater level of neurogenesis, greater numbers of neuropeptide Y and parvalbumin positive interneurons, diminished aberrant

mossy fiber sprouting, and reduced hypertrophy of astrocytes and activated microglia. Thus, SVZ-NSC grafting early after SE into the hippocampus promotes enduring control of SRS and co-morbidities with curbs on multiple epileptogenic changes and inflammation that ensue SE.

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Poster

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Topic: A.02. Postnatal Neurogenesis

Support: the NIH-MBRS-RISE Program # S06 GM59298

Title: The effects of caloric restriction on neurogenesis

Authors: *A. CARBAJAL[1], M. Y. CALAMUCHA[2], M. FUSE[2], C. MOFFATT[2], C. DULDULAU[2];
[2]Biol., [1]San Francisco State Univ., San Francisco, CA

Abstract: Adult neurogenesis occurs throughout the lifespan of many species, including vertebrates and invertebrates. The factors that regulate this process are comparatively poorly understood. To this end, the goal of this project was to determine how caloric restriction affected the rate of cell proliferation in the brain of an invertebrate, the house cricket, Acheta domesticus. Adult neurogenesis in this species occurs mushroom bodies, the insect analog of the hippocampus. Blocking neurogenesis in mushroom bodies impairs the performance of crickets in olfactory learning and memory tasks. Our studies determined how acute caloric restriction affected the rate of cell proliferation in the mushroom bodies. Crickets were starved for four days and then injected with bromodeoxyuridine (BrdU) to label proliferating cells and were then euthanized two hours later. We found that four days of starvation did not affect the rate of cell proliferation: The number of BrdU-immunoreactive cells present in the mushroom bodies did not differ significantly between freely feeding crickets and those that had been starved. Continuing experiments are replicating this observation and are determining whether or not starvation affects the survival of newly generated neurons. Elucidating how neurogenesis is regulated in crickets, and understanding the ways in which it is similar and different from that in mammals, has great potential to improve our understanding of a process that plays such an important role in learning and memory, as well in some neuropathologies.

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Poster

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Topic: A.02. Postnatal Neurogenesis

Support: P0IHL090554

Title: Chronic intermittent hypoxia suppresses adult neurogenesis and disrupts synaptic plasticity in the dentate gyrus of the hippocampus

Authors: ***M. A. KHUU**[1], C. M. PAGAN[1,2], M. LESLIE[1], J. M. RAMIREZ[1,2,3], A. J. GARCIA, III[1];
[1]Seattle Childrens Res. Inst., Seattle, WA; [2]Dept. of Pathology, [3]Dept. of Neurolog. Surgery, Univ. of Washington, Seattle, WA

Abstract: When left untreated, sleep disordered breathing (SDB) leads to significant cognitive decline. SDB is associated with chronic intermittent hypoxia (CIH), which is known to cause cognitive deficits and impaired synaptic plasticity in the CA1 neuronal population of the hippocampus. Here we studied the impact of CIH on neurophysiological processes within the dentate gyrus (DG) another key neuronal population within the hippocampal network important for integrating limbic information. Experiments were conducted in the DG of hippocampal brain slices from mice (P60-P80) left unexposed or exposed to CIH for 30days. Using immunohistological analyses, CIH did not alter structural volumes within the DG. However, CIH significantly stimulated the number of intermediate progenitors as represented by the increased number of Ki-67 positive cells within the DG region. Interestingly, the number of doublecortin positive granule neurons was significantly smaller following CIH. Thus, while CIH appears to stimulate the neuroprogenitor pool, it suppresses the transition of neuroprogenitors to newly born DG neurons. Electrophysiological studies demonstrate that CIH affects synaptic plasticity expressed in the DG. Specifically, CIH (1) decreased the degree of paired pulse facilitation and (2) disrupts LTP evoked by high frequency stimulation. We conclude that unlike many other conditions associated with disturbed oxygen homeostasis, CIH differentially influences adult neurogenesis during specific stages of development and leads to impaired synaptic plasticity. We hypothesize that these cellular effects contribute to memory deficits associated with SDB.

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Poster

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Topic: A.02. Postnatal Neurogenesis

Support: NSF Cooperative Agreement Award EPS-1003907

Title: Low-level manganese and nanosilver alter adult neural stem cell morphology and gene expression during differentiation

Authors: R. J. COOPER, A. PARSONS-WHITE, A. RAMIREZ, *N. SPITZER;
Dept of Biol. Sci., Marshall Univ. - Biol. Sci., Huntington, WV

Abstract: Heavy metals are well known for their cytotoxic and neurotoxic effects. Often, however, exposures at environmentally relevant levels are lower than those resulting in cell death. The changes in cellular mechanisms resulting from such exposure are poorly understood for two emerging environmental contaminants, manganese (Mn) and silver nanoparticles (AgNPs). Excessive environmental exposure to Mn, a micronutrient, results in cognitive deficits and neuropsychological abnormalities. AgNPs, are increasingly incorporated in industrial and consumer products due to their antimicrobial properties; they persist in the environment, bioaccumulate, and are toxic to eukaryotic systems at high concentrations. Though symptoms of over-exposure are well defined, the mechanisms underlying these effects are not as well understood. Cultured adult neural stem cells (NSC) from the subventricular zone (SVZ) of young-adult rats provide an accessible model to investigate the responses of cellular mechanisms underlying proliferation, migration, and neural differentiation to Mn or AgNP. Adult neurogenesis contributes to learning, memory consolidation, and repair, in addition to recapitulating cellular processes occurring during neurodevelopment. Previously, we found that exposure to low-level (1μg/mL) AgNPs induced the formation of f-actin inclusions in differentiating NSCs, indicating disruption of the cytoskeleton. Further, neurite extension and arborization, cytoskeleton-driven processes vital for neurogenesis, were significantly impaired. In our current study, we cultured NSC from the SVZ of rats as neurospheres and exposed them to $MnCl_2$ (10-200μM) or AgNP (1μg/mL). Cells were allowed to differentiate for 48 hours and then fixed and stained for morphology or collected for analysis of gene expression patterns by RT-PCR. We found a dose-dependent decrease in the ratio of neural to glial morphotypes following

exposure to Mn. Further, Mn upregulated GPx1, an antioxidant enzyme, and down-regulated MAP2, a neural fate marker. These data suggest that Mn exposure inhibits neuronal differentiation, and indicate oxidative stress as a possible mechanism for these effects. AgNP exposure, caused a down-regulation of DCX and Nestin, both markers of NSC proliferation. Together, these results indicate that both Mn and AgNPs, at environmentally relevant concentrations, disrupt neurogenesis by interfering with proliferation and differentiation of NSCs. Environmental exposure to these heavy metals could therefore lead to losses in learning and memory and interfere with normal neural development.

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Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

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NIH DA 016765

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Title: Galactic cosmic radiation (28Si) reduces dentate gyrus neurogenesis in the long-term in a dose-dependent manner

Authors: *A. K. WALKER[1], C. W. WHOOLERY[1], D. R. RICHARDSON[1], R. P. REYNOLDS[1], P. D. RIVERA[1], H.-Y. SHIH[2], R. L. REDFIELD[1], M. J. LUCERO[1], D. H. BEDDOW[1], S. MUKHERJEE[1], B. P. C. CHEN[2], A. J. EISCH[1];
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Abstract: Astronauts undertaking future voyages to Mars will be exposed to chronic low doses of cosmic galactic space radiation containing many different high-energy, high-charge (HZE) particles. Certain HZE particles, such as ^{56}Fe, have previously been shown to reduce the

generation of new neurons in the adult hippocampus dentate gyrus (DG), a brain region involved in memory and mood control. Diminished levels of DG neurogenesis could therefore negatively influence astronaut cognitive abilities and pose a risk to mission success. While significant data support that ^{56}Fe particles damage mouse brain and behavior, less is known about whether additional HZE particles, such as ^{28}Si, are similarly damaging. In order to fill this knowledge gap, 9-week old mice were exposed to whole-body ^{28}Si particle ground-based radiation at doses of 0 cGy (SHAM), 20 cGy, and 100 cGy at Brookhaven National Laboratories. DG neurogenesis was evaluated at two time points after irradiation (IRR) in order to assess short-term (24-hours) versus long-term (3-months) effects. To label dividing cells, mice were given a single BrdU injection 22 hours post-IRR and perfused either 2 hours or 3 months later. Using immunohistochemistry (IHC) and sterological approaches, tissue from the 24-hour group was labeled for markers of proliferation (BrdU and Ki67), and immunoreactive cells were quantified. In the 24-hour group, mice exposed to 100 cGy had fewer DG BrdU+ and Ki67+ cells as compared to SHAM, revealing a significant decrease in proliferation. Additionally, in both the 24-hour group and 3-month group, quantification of doublecortin immunoreactive (DCX+) cells was performed to assess levels of neurogenesis. In the 24-hour group, mice exposed to 20cGy and 100cGy IRR displayed a dose-dependent reduction in DG DCX+ cells compared to SHAM. However, in the 3-month group, only mice exposed to 100cGy IRR had fewer DG DCX+ cells compared to SHAM. These data suggest ^{28}Si radiation dose-dependently decreases neurogenesis in the short-term, but that there may be recovery of neurogenesis in the long-term in mice that received the lower dose of IRR. Additional studies on the long-term influence of ^{28}Si IRR on neurogenesis, cell morphology and death, and behavior are underway. This work is part of a broader effort to gain better understanding of whether space radiation is detrimental to the brains of future deep space faring astronauts.

Disclosures: **A.K. Walker:** None. **C.W. Whoolery:** None. **D.R. Richardson:** None. **R.P. Reynolds:** None. **P.D. Rivera:** None. **H. Shih:** None. **R.L. Redfield:** None. **M.J. Lucero:** None. **D.H. Beddow:** None. **S. Mukherjee:** None. **B.P.C. Chen:** None. **A.J. Eisch:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 664.27/B24

Topic: A.02. Postnatal Neurogenesis

Title: Effects of castration on adult hippocampal neurogenesis

Authors: *K. ATKINSON[1], T. ALKAM[1], S. DIAZ[2], A. ROBBINS[3], R. N. PECHNICK[1]; [1]Western Univ. of Hlth. Sci., Pomona, CA; [2]California State Polytechnic Univ., Pomona, CA; [3]Pitzer Col., Claremont, CA

Abstract: Androgen-deprivation therapy is a common treatment for prostate cancer. Its goal is to lower the level and/or effects of testosterone, a critical factor driving the progression of the disease. However, patients subjected to these treatments frequently exhibit symptoms of cognitive impairment. The relationship between androgen-deprivation and cognitive impairment is poorly understood. We propose that the cognitive impairment is due to deficits in hippocampal neurogenesis. The purpose of this study was to test the hypothesis that adult hippocampal neurogenesis is disrupted after castration-induced decreases in plasma testosterone levels in mice. Seven week-old, male C57/BL6 mice underwent either castration or sham surgery. In the castration surgery, both testes were removed, whereas in the sham surgery the mice received an incision in the same area but the testes were not removed. The survival of the proliferating neurons was assessed using 5-bromo-2-deoxyuridine (BrdU). One week after surgery, mice were injected with BrdU (50 mg/kg i.p.) every 2h for a total of four times. Four weeks later, the mice were anesthetized and sacrificed by cardiac perfusion, fixed with 4% paraformaldehyde, the brains sremoved and immunohistochemical studies were carried out. We found that there was a decrease in the number of BrdU-labeled cells in the dentate gyrus of the hippocampus in the castrated mice compared to the sham mice. We also found that there was a decrease in the number of cells expressing Ki-67, a marker for cell proliferation. The results suggest that testosterone is critical for normal neuronal proliferation and survival in the hippocampus and support our contention that cognitive impairment after androgen-deprivation therapy might be a consequence of disruption of hippocampal neurogenesis.

Disclosures: K. Atkinson: None. **T. Alkam:** None. **S. Diaz:** None. **A. Robbins:** None. **R.N. Pechnick:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 664.28/B25

Topic: A.02. Postnatal Neurogenesis

Support: NIH/NIDA Grant R01DA021249

Title: Effects of prenatal and/or adolescent exposure to nicotine on hippocampal neurogenesis in adult rats

Authors: *T. ALKAM, S. O'DONNELL, R. N. PECHNICK;
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Abstract: Exposure to tobacco smoke (nicotine) prenatally or during adolescence increases the likelihood of suffering cognitive impairment in adult life. Some drugs of abuse are known to affect adult hippocampal neurogenesis; however, the relationship between developmental exposure to nicotine and adult hippocampal neurogenesis is poorly understood. The purpose of the current study was to define the effects of prenatal or/and adolescent nicotine exposure on hippocampal neurogenesis and begin to identify molecular pathways underlying the effects. On day 4 of pregnancy the dams were implanted with subcutaneous osmotic mini-pumps containing either vehicle (sodium bitartrate in saline) or nicotine bitartrate (6.0 mg/kg/day). During the adolescent period (postnatal days 33-40) male pups from the vehicle- and nicotine-treated dam received injections of either saline or nicotine (0.4 mg/kg/s.c.) for 8 consecutive days. On postnatal day 150, the rats were sacrificed and brains were collected for Western blot and immunohistochemistry studies. Analysis of Western blots showed that the expression of Ki67, a marker of cellular proliferation, and doublecortin, a microtubule-associated protein expressed by neuronal precursor cells and immature neurons, were significantly increased in the hippocampus of the rats that received both prenatal and adolescent nicotine exposure. On the other hand, the protein level of calbindin, a marker for mature neurons, was significantly down-regulated. The current results show that combined prenatal and adolescent nicotine exposure might have long-term effects on adult hippocampal neurogenesis.

Disclosures: T. Alkam: None. **S. O'Donnell:** None. **R.N. Pechnick:** None.

Poster

664. Postnatal Neurogenesis: Environmental and Pharmacologic Regulation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 664.29/B26

Topic: A.02. Postnatal Neurogenesis

Support: Spanish Ministerio de Ciencia e Innovacion (MICINN)

Spanish Ministerio de Economía y Competitividad (MINECO)

Instituto de Salud Carlos III (ISCIII; CIBERNED)

Comunidad de Madrid, Spain

Title: Role of systemic and local insulin-like growth factor-I in the regulation of the sequential stages of postnatal/adult hippocampal neurogenesis

Authors: *C. VICARIO-ABEJON[1], V. NIETO-ESTEVEZ[1], C. O. OUESLATI-MORALES[1], J. PICKEL[2];
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Abstract: Insulin-like growth factor-I (IGF-I) is involved in the regulation of adult hippocampal neurogenesis, although its specific actions have not been fully defined. Moreover, the role of locally-produced IGF-I in adult neurogenesis has not been addressed. We show that both IGF-I and IGF-IR are expressed in the postnatal/adult dentate gyrus (DG). To study the influence of IGF-I on the stages of DG neurogenesis, we analyzed sections from postnatal/adult global Igf-I KO mice (Igf-I-/- and Igf-I+/+ mice) and from a nervous system specific Igf-I conditional KO (Igf-IΔ/Δ and Igf-ICtrl mice). In both KO mice we found an accumulation of progenitor cells, some of which were situated ectopically in the outer granule cell layer (GCL) and in the molecular layer (ML). Indeed, the GCL was disorganized in both KO animals, with significantly more Prox1+ granule neurons outside this layer than in WT mice. Dividing progenitors were also generated in higher numbers in clonal neural stem cell cultures prepared from the Igf-I-/- hippocampus. To determine the impact of Igf-I deletion on newly formed neurons *in vivo*, dividing progenitors in P21 Igf-I-/- and Igf-I+/+ mice were labeled with GFP-expressing retroviral particles. This revealed that in the Igf-I-/- mice more GFP+ cells expressed markers of immature neurons and they had less complex dendritic trees. Consequently, there were fewer mature granule (calbindin+ cells) in the Igf-I-/- animals. Our findings indicate that IGF-I plays critical roles during postnatal/adult DG neurogenesis, regulating in a paracrine manner the number of progenitors as well as the positioning and the molecular and morphological differentiation of newly formed neurons.

Disclosures: C. Vicario-Abejon: None. **V. Nieto-Estevez:** None. **C.O. Oueslati-Morales:** None. **J. Pickel:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.01/B27

Topic: A.03. Stem Cells

Title: Characterization of NMDA receptors in human induced pluripotent stem cell-derived neurons

Authors: H. FUKUI[1], *H. VON DER KAMMER[1], I. NEAGOE[1], A. STUMPF[2], Y. LU[3], D. HE[3], R. FRANCIS[3], J. CHEN[3], P. REYNEN[3], M. ALAOUI-ISMAILI[3];
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Abstract: Aberrant synaptic transmission mediated by N-methyl-D-aspartate receptors (NMDARs) has been implicated in various neuropathological conditions ranging from psychiatric to neurodegenerative disorders. Previous efforts to develop novel modulators of NMDARs utilizing heterologous expression systems, however, have led to little success in developing clinically tolerable, efficacious chemical matter. To assess the utility of human induced pluripotent stem cell (hiPSC)-derived neurons developed by Cellular Dynamics International (hereafter, iCell® Neurons) for *in vitro* validation of NMDAR modulators, we initially confirmed the expected electrophysiological responses of iCell® Neurons to glutamate and NMDA by using manual patch-clamp recordings. Assessment of the relative expression of NMDAR subunits by qRT-PCR revealed the highest expression of NR2B, medium expression of NR1 and NR2D, and low or no expression of NR2A and NR2C transcripts in these cells. The relative abundance of NR2 transcripts did not markedly change from Day 3 to Day 15 post-plating, which was confirmed by Western blot analysis. Manual patch-clamp assessment of iCell® Neurons using various pharmacological tools also pointed out the predominance of NR2B among the other NR2 subunits; IC50 determination of the NR2B selective antagonist ifenprodil against NMDA-induced currents revealed a similar potency of ifenprodil in iCell® Neurons and human NR2B-expressing recombinant cell lines. Taken together, these findings suggest that iCell® Neurons predominantly express functional NR2B-containing NMDARs and could serve as a valuable system for development and validation of NR2B-modulating pharmaceutical agents.

Disclosures: **H. Fukui:** A. Employment/Salary (full or part-time):; Evotec AG. **H. Von Der Kammer:** A. Employment/Salary (full or part-time):; Evotec AG. **I. Neagoe:** A. Employment/Salary (full or part-time):; Evotec AG. **A. Stumpf:** None. **Y. Lu:** A. Employment/Salary (full or part-time):; Genentech. **D. He:** A. Employment/Salary (full or part-time):; Genentech. **R. Francis:** A. Employment/Salary (full or part-time):; Genentech. **J. Chen:** A. Employment/Salary (full or part-time):; Genentech. **P. Reynen:** A. Employment/Salary (full or part-time):; Genentech. **M. Alaoui-Ismaili:** A. Employment/Salary (full or part-time):; Genentech.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.02/B28

Topic: A.03. Stem Cells

Support: MBI

NRF Grant NRF-CRP002-082

Title: Topographical influence on differentiation of pluripotent stem cells into regionalized dopaminergic neurons

Authors: ***K. K. TAN**[1], W. LIM[2], C. CHAI[2], K. LIM[2], E. L. K. GOH[2], E. K. F. YIM[1]; [1]Mechanobiology Inst., Singapore, Singapore; [2]Duke-NUS Grad. Med. Sch. Singapore, Singapore, Singapore

Abstract: Parkinson's disease is a neurodegenerative disease attributed to the loss of midbrain dopaminergic (DA) neurons. Pluripotent stem cells hold great promise in the study and clinical treatment for this neurodegenerative disease but progress has been hampered by the acquirement of robust cells, posing a major barrier to drug development. Appropriate biophysical cues can direct stem cell fate but the role of topography in differentiating stem cells into subtype specific cells has hitherto not been well understood. We aim to develop an *in vitro* substrate that will accelerate the derivation of midbrain dopaminergic (DA) neurons from human pluripotent stem cells (hPSCs). By using human induced pluripotent stem cells (iPSCs), we have examined the ability of topographical patterns to influence the differentiation of hPSCs into subtype-specific and regionalized dopaminergic (DA) neurons. We have made minor modifications to the protocol based on dual SMAD inhibition method and optimized on patterned substrates to further improve the efficiency for DA neuron derivation. The size of embryoid bodies was standardized on fabricated PDMS chambers consisting of microwells. We have also optimized the use of region specific markers to study regionalized specification of DA neurons on patterned substrates. Our current progress in using patterned substrates for DA differentiation will be presented here. It is hoped that this will also provide novel insights into mechanisms underlying DA neuronal development and ultimately discover new therapeutic approaches for this neurodegenerative disease.

Disclosures: **K.K. Tan:** None. **W. Lim:** None. **C. Chai:** None. **K. Lim:** None. **E.L.K. Goh:** None. **E.K.F. Yim:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.03. Stem Cells

Support: MEXT KAKENHI 26670545

Title: Influence of carbonyl stress on neural cells derived from induced pluripotent stem cell

Authors: ***Y. HORIUCHI**, N. OBATA, I. NOHARA, A. KOBORI, K. TORIUMI, M. ITOKAWA, M. ARAI;
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Abstract: Schizophrenia (SZ) is a devastating mental illness in which initial and major risks of the disease during neurodevelopment may disturb postnatal brain maturation, which results in the onset after puberty. However, mechanistic understanding of mental disorders, such as schizophrenia and bipolar disorder, is not well developed. One major limitation that has blocked the progress is the difficulty of accessing relevant tissues/cells for the investigation. First, although mental disorders affect the brain, it is almost impossible to obtain biopsied brains or neurons of central nervous system origin that are relevant to the diseases. Second, because the onset of these disorders is relatively young (adolescence or young adulthood), there is no guarantee that autopsied brains from aged patients with long-term medication reflect disease pathologies. For these reasons, there is expectation that induced pluripotent stem cells (iPS cells) will be a major advance for understanding of mental disorders. Astrocytes facilitate neuronal maturation by regulating exogenous stress. Antioxidant defense is one example of this type of astrocyte function. Previous study from our laboratory reported that the carbonyl stress in a subpopulation of SZ patients, leading to a failure of metabolic systems with plasma pentosidine accumulation and serum pyridoxal depletion. However the molecular mechanisms of the relationship between carbonyl stress and SZ are still unknown. We hypothesize that astrocytes may have a deficit in energy metabolism resulting in neuronal damage in SZ, which might be involved in SZ pathology in carbonyl stress context. As a first step of the study, we examined how pentosidine accumulation affects to neuron and astrocyte using human iPS cells. We generated TUJ1 positive neurons and GFAP positive astrocyte via neurosphere formation from human iPS cells of normal control subjects. And then we measured the energy metabolism such as glycolysis and mitochondrial respiration in the cells. Our strategy will provide important clues for understanding of SZ.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.03. Stem Cells

Support: NIH Grant 1R01MH100900

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NIH Grant F30MH106261

NIH Grant 5R37MH060233

NIH Grant 5R01MH094714

Title: Generating functional cortical neurons and astrocytes from human pluripotent stem cells in 3D cultures

Authors: *S. A. SLOAN[1], A. M. PASCA[1], L. E. CLARKE[1], Y. TIAN[2], C. D. MAKINSON[1], N. HUBER[1], J.-Y. PARK[1], C.-H. KIM[3], N. O'ROURKE[1], K. D. NGUYEN[1], S. J. SMITH[4], J. R. HUGUENARD[1], D. H. GESCHWIND[2], B. A. BARRES[1], S. P. PASCA[1];
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Abstract: Human cortical development consists of intricate cellular processes that are coordinated across time and space. Protocols for inducing neural differentiation from pluripotent stem cells *in vitro* have provided unique opportunities to study features of normal and abnormal corticogenesis, but current techniques are largely limited to two-dimensional systems that lack the cellular maturity and three-dimensional (3D) cytoarchitecture typically found in the developing cortex. Here, we present a simple and reproducible 3D culture approach for generating a laminated cerebral cortex-like structure, named human cortical spheroids (hCS), from human pluripotent stem cells. hCS are easy to maintain in culture and are reproducible within and across differentiations. hCS contain both deep and superficial layer cortical neurons and can be mapped transcriptionally to *in vivo* human cortical developmental stages up to late mid-fetal periods. Neurons are electrophysiologically mature, display spontaneous activity, are surrounded by non-reactive astrocytes, and form functional synapses. Experiments in acute slices of hCS demonstrate that cortical neurons participate in network activity and produce complex synaptic events. These 3D cultures allow a detailed interrogation of human cortical development,

function and disease, and represent a versatile platform for generating other neuronal and glial subtypes *in vitro*.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.05/B31

Topic: A.03. Stem Cells

Support: 5-T32-NS007180-30

R01-NS-56243-05

R01-NS-38690-11

Title: Induction of neural stem cell migration through transposon-mediated reprogramming

Authors: *F. SIDDIQI[1], A. L. TRAKIMAS[2], R. RISBUD[2], E. D. MARSH[3], J. H. WOLFE[3]; [1]Dept. of Neurol., [3]Neurol., [2]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Neural stem cell (NSC) transplantation therapy can serve as a therapeutic model in damaged or diseased brains. Often NSC lines have been selected based upon their endogenous ability to engraft into host tissue. In contrast, when primary immune-compatible NSCs are used, their ability to migrate is significantly attenuated. However, subsets of primary neural progenitor cells (NPCs) show extensive migration during brain development, such as neural crest and medial ganglionic eminence (MGE) cells. It is currently unknown whether or not NSCs can be reprogrammed from non-migratory into migratory states using upstream transcription factors (TFs). In the current study, we conducted a meta-analysis of micro-array data sets comparing these cell populations at active stages of migration to identify transcription factors (TFs) associated with migratory pathways. We conducted DAVID functional clustering analysis and identified several candidate genes that were differentially expressed during migratory stages. To functionally test candidate TFs, we generated piggyBac (PB) vectors for 5 TF conditions, which allowed for ectopic expression of candidate TFs in non-migratory NSCs. Our results showed that at least 2 candidate TFs were able to induce migration in *in vitro* scratch assays compared to

control condition. These cells gained bipolar morphologies with dynamic growth cones consistent with cell motility. *In vivo* transplantation of 1 TF condition into neonatal mice brains resulted in increased engraftment. Our results suggest upstream TFs are able to induce NSC migration for utility in transplantation therapy.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.06/B32

Topic: A.03. Stem Cells

Title: Differentiation of midbrain floor plate progenitors and dopaminergic neurons from human pluripotent stem cells

Authors: *S. SHIN, M. DERR, Y. YAN, L. SANGENARIO, K. VEDVIK, A. HANNAY, D. KUNINGER;
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Abstract: Midbrain dopaminergic (DA) neurons derived from human pluripotent stem cells (hPSCs) provide an excellent source for disease modeling and drug screening for Parkinson's disease. During brain development, midbrain floor plate (mFP) is formed during 21-28 days of gestation along the ventral midline of developing neural tube and it has been shown that midbrain DA neurons are differentiated from mFP cells. Recent reports have focused on identifying the appropriate *in vitro* conditions to differentiate hPSCs to properly regionalized floor plate precursors, rather than a more general neural stem cell population, in order to create authentic DA neurons. However, published protocols are quite lengthy and complicated leading to increased variability in differentiation efficiencies. Also, few reports describe whether specified progenitors can be expanded and cryopreserved. Our objective is to develop a culture media system designed to simplify and standardize this process while compressing timelines and adding increased flexibility in this complex differentiation workflow. Here we describe our results which have broken the process down into 3 distinct steps: (1) specification of hPSC to midbrain floor plate (mFP) cells, (2) expansion and cryopreservation of derived mFP cells, and (3) maturation to DA neurons. Characterization of floor plate cells and mature DA neurons was performed by immunostaining for the presence of specific markers including Lmx1, Otx2, FoxA2 and TH, additional qPCR analysis included expanded lists of genes to help define these

cell populations. Electrophysiological characteristics of differentiated neurons were assessed by Multi-electrode array and spontaneous and depolarization induced dopamine release was measured with HPLC. In comparison to published protocols, our new system has several advantages including ease of use, significant expansion and preservation of progenitors in relatively short culture duration. This efficient system will benefit researchers with increased scale and flexibility in targeted studies.

Disclosures: **S. Shin:** A. Employment/Salary (full or part-time):; Thermofisher scientifics. **M. Derr:** A. Employment/Salary (full or part-time):; Thermofisher scientifics. **Y. Yan:** A. Employment/Salary (full or part-time):; Thermofisher scientifics. **L. Sangenario:** A. Employment/Salary (full or part-time):; Thermofisher scientifics. **K. Vedvik:** A. Employment/Salary (full or part-time):; Thermofisher scientifics. **A. Hannay:** A. Employment/Salary (full or part-time):; Thermofisher scientifics. **D. Kuninger:** A. Employment/Salary (full or part-time):; Thermofisher scienfics.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.07/B33

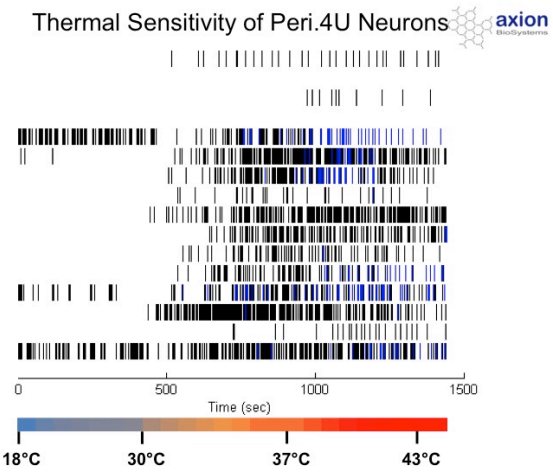
Topic: A.03. Stem Cells

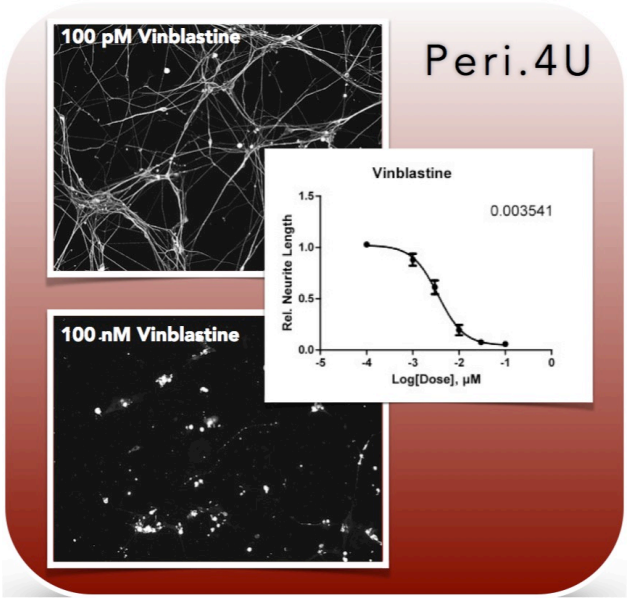
Title: Human iPSC-derived neurons: ideal for electrophysiological and toxicological assays

Authors: *B. MURPHY[1], G. LUERMAN[1], A. EHLICH[2], T. PALM[2], A. DUENBOSTELL[2], R. KETTENHOFEN[2], A. NICOLINI[3], B. BADER[4], A.-M. PIELKA[4], C. EHNERT[4], A. VOSS[4], O. SCHRÖDER[4], H. BOHLEN[2];
[1]Axiogenesis Inc, Plymouth Meeting, PA; [2]Axiogenesis AG, Cologne, Germany; [3]Axion Biosystems, Atlanta, GA; [4]NeuroProof GmbH, Rostock, Germany

Abstract: New standards are needed to address drug development for neurological degenerative diseases and neurotoxicological liability screens. Current drug development and toxicology screens employ classical animal derived *in vitro* and *in vivo* models, but lack true human neuronal cells. Axiogenesis developed different types of human induced pluripotent stem cell (iPSC) derived neurons (dopaminergic and peripheral neurons) to address the lack of relevant human central nervous as well as peripheral cellular systems. Here we demonstrate the suitability of these models in various neurotoxicological assays, such as: 1) Functional synaptic network activity of dopaminergic and peripheral neurons plated in 12 well microelectrode arrays. While dopaminergic neurons showed burst patterns similar to primary midbrain neurons from mouse,

peripheral neurons demonstrated increased thermal sensitivity; 2) Within a neurite outgrowth assay, our peripheral neurons highlighted enhanced sensitivity to chemotherapeutics (e.g. Vinblastine) than the gold-standard rat PC12 “neurite” cell model. Together, these data show that Axiogenesis human iPSC-derived neuronal subtypes are relevant human cell models and are ideal for a variety of well-established toxicological assays.





Disclosures: **B. Murphy:** None. **G. Luerman:** None. **A. Ehlich:** None. **T. Palm:** None. **A. Duenbostell:** None. **R. Kettenhofen:** None. **A. Nicolini:** None. **B. Bader:** None. **A. Pielka:** None. **C. Ehnert:** None. **A. Voss:** None. **O. Schröder:** None. **H. Bohlen:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

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Topic: A.03. Stem Cells

Support: NIH Grant HL 113905

President's Faculty Research Development Grant NSU

Title: Selective stimulation of AT2 angiotensin II receptor subtype increases neural stem cell proliferation

Authors: B. BLANCO[1,2], L. COULING[3], P. PATEL[4], S. KAMISETTY[4], M. TRIVEDI[3], J. MUNOZ[4], *R. C. SPETH[3,4,5];
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Abstract: Activation or inhibition of the renin-angiotensin system (RAS) affects neuronal function and viability. To assess the effect of angiotensin II (Ang II) receptor stimulation on neuronal growth and development, human neuronal stem cells (H-9 derived, Life Technologies), which were shown by qRT-PCR to express detectable levels of mRNA for components of the RAS, were seeded in chamber slides and cultured under proliferation (with EGF and FGF-2) or differentiation (no added growth factors) conditions with chronic exposure to AT1 and AT2 selective agonists. Conditions included 14 days treatment with once daily addition of the AT2 selective agonist CGP42112 (final concentration 100 nM); once daily treatment with the non-selective Ang II receptor agonist Sar1 angiotensin II (100 nM) plus the AT2 receptor selective antagonist PD123319 (10 μM) for selective AT1 receptor stimulation; once daily treatment with PD123319 (10 μM) alone; or once daily treatment with vehicle. Following treatment, cells were immunostained for the proliferation marker PCNA; the neural stem/progenitor cell marker Nestin; the neuroblast marker doublecortin; the astrocyte marker GFAP; the neuronal marker HuCD, and the DNA counterstain DAPI. Two-way ANOVA assessment of DAPI counts of cells revealed that there was a higher density of cells, as revealed by DAPI staining, in the proliferation medium compared to the differentiation medium ($p<0.0001$). There was a significant treatment effect ($p=0.0026$). The AT2 selective agonist CGP42112 significantly increased the density of cells compared to the AT1, PD1123319 and control groups ($p<0.05$) regardless of growth conditions. There were no significant differences between AT1 stimulated, PD123319 treated and the control groups, regardless of growth conditions. Preliminary results from immunostaining are consistent with an increase in cell growth with AT2 treatment with CGP42112. These results suggest that AT2 receptor stimulation can promote neural stem cell proliferation in both proliferation and differentiation conditions.

Disclosures: **B. Blanco:** None. **L. Couling:** None. **P. Patel:** None. **S. Kamisetty:** None. **M. Trivedi:** None. **J. Munoz:** None. **R.C. Speth:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.09/B35

Topic: A.03. Stem Cells

Support: NIH Intramural funds

Title: Cell type and species specific toxicity screen of human neural stem cells and rat cortical neurons

Authors: *J. P. STEINER[1], A. G. EFTHYMIOU[2], K. MATHER[1], N. CHESTER[1], X. WANG[2], M. RAO[2], N. MALIK[2], A. NATH[1];
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Abstract: Human primary neural tissue is a vital component for the quick and simple determination of chemical compound neurotoxicity *in vitro*. In particular, such tissue would be ideal for high-throughput screens that can be used to identify novel neurotoxic or neurotherapeutic compounds. We have previously established a high-throughput screening platform using human induced pluripotent stem cell (iPSC)-derived neural stem cells (NSCs) and neurons. In this study, we conducted a 2000 compound screen with human NSCs and rat cortical neuronal cells to identify compounds that are selectively toxic to each group. Approximately 100 of the tested compounds showed specific toxicity to human NSCs when screened at a concentration of 10 micromolar. A confirmatory and secondary screen of a small subset of compounds from the primary screen on human iPSCs, NSC-derived neurons, and fetal astrocytes validated the results from more than 80% of these compounds, with some showing cell specific toxicity. Amongst those toxic compounds were several cardiac glycosides, all of which were selectively toxic to the human cells. As the screen was able to reliably identify neurotoxicants, many with species and cell-type specificity, this study demonstrates the feasibility of this NSC-driven platform for higher-throughput neurotoxicity screens.

Disclosures: **J.P. Steiner:** None. **A.G. Efthymiou:** None. **K. Mather:** None. **N. Chester:** None. **X. Wang:** None. **M. Rao:** None. **N. Malik:** None. **A. Nath:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.10/B36

Topic: A.03. Stem Cells

Support: Wellcome Trust ISSF Grant (No. 097819)

King's Health Partners

Royal Society UK

The Brain and Behavior Foundation (formally National Alliance for Research on Schizophrenia and Depression (NARSAD))

Title: Exploring the role of estrogen in early corticogenesis using human induced pluripotent stem cells

Authors: *C. SHUM[1], S. MACEDO[2], K. WARRE-CORNISH[2], D. P. SRIVASTAVA[2]; [2]Basic and Clin. Neurosci., [1]King's Col. London, London, United Kingdom

Abstract: Estrogens have a mechanistic influence during critical periods of prenatal brain development. The actions of estrogens are mediated by multiple neuronal signalling pathways, involving classical and non-classical estrogen receptors (ERs). The “classical” pathway involves estrogens binding to ERα and ERβ, transcription factors that regulate target gene transcription via binding to estrogen response element sequences and recruitment of other regulatory proteins. The “non-classical” pathway involves estrogens binding to membrane-bound ERα and ERβ, as well as the G protein-coupled receptor GPER1. Activation of these receptors results in the activation of multiple effectors, leading to various downstream effects. The activation of these pathways in neurons has been shown to promote survival, neuritogenesis and modulation of synaptic function. Interestingly, sex differences have been observed in the distribution of estrogen receptors in some brain regions and males and females and males respond differently to estrogen treatment in the brain. The expression and function of ERs and related genes in neurodevelopment are not well known. In addition, it is not clear which cellular and subcellular targets of estrogens are mediating its neuroprotective and neuroreparative actions, and whether there are sex differences in these and other components of the estrogen signalling pathway. We have used human induced pluripotent stem cells (iPSCs) to explore the role of estrogens in corticogenesis. Real time PCR and luciferase assays were used to examine the expression and function of estrogen receptors and related genes during the differentiation of iPSCs to cortical

projection neurons. Immunocytochemistry and live cell imaging were also used to characterise the effect of estradiol and estrogen receptor agonists on neuronal morphology and the expression of synaptic proteins in cortical projection neurons differentiated from iPSCs. We observed expression of estrogen receptors during neural induction and maturation. We have found that estradiol and estrogen receptor agonists have differential effects on neurite outgrowth. Our findings show that the neuritogenic actions of estrogen are mediated by specific components of the estrogen signalling pathway and support the use of human induced pluripotent stem cells for the study of the molecular mechanisms underlying the mechanistic influence of estrogen.

Disclosures: C. Shum: None. **S. Macedo:** None. **K. Warre-Cornish:** None. **D.P. Srivastava:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.11/B37

Topic: A.03. Stem Cells

Title: Skip (stemcell knowledge and information portal). One stop database for researchers, commercial entities and citizens

Authors: *T. KONDO[1,2], S. KAWASE[2], J. TSUYAMA[2], T. SHIMURA[2], K. FUJIMORI[2], S. SUZUKI[2], S. ITO[2], M. TSUJIMOTO[2], K. KOSAKI[2], T. MASUI[2];
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Abstract: In 2014 Japanese government launched three Acts in the field to promote and secure regenerative medicine. Therefore, the 2014 is called the first year of regenerative medicine in Japan. Acceleration of stem cell sciences and realization of their outcomes to benefit patients, efficient exchange of stem cells information and inspiring database for science are indispensable. This database also aims to bridge science and patients and citizens to facilitate their participation in clinical research. SKIP is an initiative to promote the exchange of information and facilitate joint research between researchers by providing one stop database of information of stem cells (iPS cells, original diseased fibroblasts, lymphoblast cells, etc.), data include cell types, ownership, characters, culture and preservation conditions, literature, etc.. SKIP also aims to offer information on stem cells to the general public, including patients, in order to promote societal understanding and enhance participation of medical research using stem cells. SKIP

(http://www.skip.med.keio.ac.jp/) is administrated by an operating committee ("SKIP Operating Committee") of Keio University as Human Stem Cells Informatization Project, the Ministry of Health, Labour and Welfare. To date, we have had more than 550 cell information without duplication. The information is registered from published papers and open resources, and also from original establishers of their culture. We also create and provide secure database to share stem cell information between different institutions at collaborations. We are calling you to register your own cell lines to SKIP to promote your collaboration. Accession reached more than 300 per day and is increasing. Researchers can reach disease iPS cells of their interest from the name of disease or ICD-10 code. Visibility of the database increase and we are trying to add detailed relational information of cells and make the database more fruitful. SKIP is developing to be a powerful tool for researchers to liberate their ideas and promote stem cell sciences involving public and commercial entities.

Disclosures: **T. Kondo:** None. **S. Kawase:** None. **J. Tsuyama:** None. **T. Shimura:** None. **K. Fujimori:** None. **S. Suzuki:** None. **S. Ito:** None. **M. Tsujimoto:** None. **K. Kosaki:** None. **T. Masui:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.12/B38

Topic: A.03. Stem Cells

Title: Long-term electrophysiological activities and drug responses in cultured human iPSC derived neurons

Authors: ***A. ODAWARA**[1], Y. SHI[2], H. JIKO[3], I. SUZUKI[1];
[1]Tohoku Inst. of Technol., Sendai, Japan; [2]Axol Biosci. Ltd, Cambridge, United Kingdom;
[3]Alpha MED Scientific Inc., Osaka, Japan

Abstract: Neuronal cells can be generated from Human induced pluripotent stem cells (hiPSCs), providing a very important alternative to studies of humans and model organisms, to facilitate a better understanding of the mechanisms of neurological diseases and identifying novel therapeutic avenues. However, the long-term electrophysiological futures of cultured human iPSC-derived neurons have not been investigated. Here, we used the multi-electrode array system to investigate the functional characteristics of hiPSC-derived neurons on their long-term spontaneous activity and drug responsiveness. We demonstrated that hiPSC-derived neurons allowed the culture to be maintained for >260 days with long-term spontaneous activity. After 2

months of culture, we observed synchronous burst firing activity due to synapse transmission within neuronal networks. Compared with rat neurons, hiPSC-derived neurons required longer time to mature functionally. In drug responsiveness, addition of the synapse antagonist bicuculline, CNQX, AP5 and agonist a kainic acid, L-gulutamate induced significant changes of the firing rate. Furthermore, administration of pentylentetrazole (PTZ) induced epilepsy phenomenon. Anti-epilepsy drug phenytoin and sodium valproate reduced epilepsy phenomenon. These results suggested that long-term electrophysiological measurements in hiPSC-derived neurons using a MEA system may be beneficial for clarifying the functions of human neuronal circuits and for drug screening applications.

Disclosures: **A. Odawara:** None. **Y. Shi:** None. **H. Jiko:** None. **I. Suzuki:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.13/B39

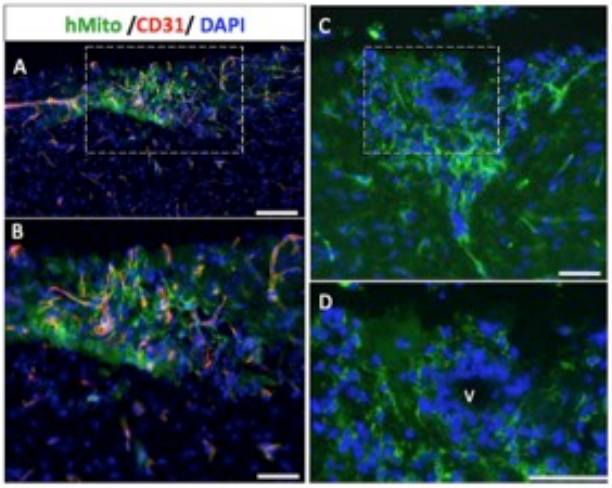
Topic: A.03. Stem Cells

Title: Development and integration of engrafted induced pluripotent stem cells-derived 3-dimensional cerebral organoids

Authors: *N. DAVIAUD, H. ZOU;
Lab. of axonal growth & neuronal regeneration, Mount Sinai Sch. of Medecine, New York, NY

Abstract: Stem cells hold great promise for modeling brain disorders and promoting neural repair, but are limited by the lack of 3-dimensional (3D) organization. Recently, 3D cerebral-organoid (C-organoid) culture system was developed, containing discrete brain structures, such as forebrain cortical plate with stereotypical inside-out stratification and regional specifications. However, C-organoids stop progressing due to nutritional limitation. To overcome this obstacle, we propose to transplant C-organoids into neonatal mouse brains to achieve *in vivo* vascularization, which may further promote morphological maturation and advance neural differentiation of the grafted organoids. In addition, formation of *in vivo* vasculature may also orchestrate signals between endothelial cells and neural progenitors, recapitulating the interaction of cells and tissues during corticogenesis. In a recent pilot study, we transplanted *in vitro* derived C-organoids and demonstrated good survival and robust *in vivo* vascularization in neonatal mouse cortex (Fig. 1). We also observed the expression of stem cell, intermediate progenitor and preplate markers. Importantly, at 1 month post-transplant, the engrafted C-organoid maintained a ventricle-like cavity, an important structure for maintaining polarity of the

germinal zone and stem cell niche. Further analysis will be performed to study proliferation and differentiation state of the implanted tissue. We propose an innovative approach using *in vitro* 3D C-organoid culture as a unique platform to model human cortical development. Once established, engraftment-based cerebral organoid studies will have far-reaching implications in illuminating human brain evolution and in advancing CNS repair strategies. Fig. 1. Grafts in motor cortex of P14 mouse after 1 month. (A, B) Immunofluorescence shows abundant vasculature (CD31, red) in grafted organoids labeled by anti-human mitochondria antibody (green). (C, D) A ventricle-like cavity (V) is preserved in the graft. Inset of A and C are enlarged in B and D. Scale: 100 μm (A), 25 μm (B-D).



Disclosures: **N. Daviaud:** None. **H. Zou:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.14/B40

Topic: A.03. Stem Cells

Support: Wellcome Trust RG53710

Wellcome Trust RG69895

Title: Probing network dynamics in an *in vitro* model of human cortex development

Authors: *M. PETER, P. KIRWAN, F. J. LIVESEY;
Gurdon Inst., Cambridge, United Kingdom

Abstract: One fundamental question in neuroscience is how neurons form specific connections and functional neuronal networks. During development, neurons typically undergo a phase of over-connectivity followed by synaptic pruning and a reduction in neuronal connectivity. Furthermore, network activity undergoes different phases of synchronous oscillatory firing before complex firing pattern emerge. The functional role of this oscillatory activity is not fully understood, however it is believed that synchronous network activity is important to shape neuronal connectivity and the development of mature neuronal networks. Due to the limitations of studying human fetal material, later aspects of cerebral cortex development has largely been studied in rodents. Our lab has previously published a method to replay cortical development, generating human cortical neurons from pluripotent stem cells. These neurons express markers from all cortical layers and develop excitatory neuronal networks that recapitulate rodent network development over months in culture. Network activity is sensitive to tetrodotoxin, and is AMPA and NMDA receptor-dependent. Therefore human stem cell derived neurons serve as an ideal model system to study human cortical network development *in vitro*. Currently I am combining imaging of neuronal network activity using GCaMP6 and tracing of neuronal connectivity with a modified rabies virus to link activity with a connectivity analysis. I am also combining trans-synaptic rabies tracing with a histological analysis of connected neurons to test if late born neurons integrate into existing neuronal networks. In the future I will further explore the contribution of inhibition on network activity development by co-culturing neurons with stem cell derived interneurons. These experiments will allow me to better understand how different activity pattern shape neuronal connectivity during human cortex development.

Disclosures: **M. Peter:** None. **P. Kirwan:** None. **F.J. Livesey:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

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Topic: A.03. Stem Cells

Support: NIH Grant P51OD011106

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Title: Common marmoset ipsc-derived midbrain floorplate neuroprogenitors

Authors: *S. C. VERMILYEA[1,2], S. GUTHRIE[2], M. MEYER[2], K. BRAUN[2], K. SMUGA-OTTO[2], S. HOWDEN[7], J. A. THOMSON[7], S.-C. ZHANG[3], T. G. GOLOS[2,4,5], M. E. EMBORG[1,2,6];
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Abstract: Neuronal differentiation for *in vitro* disease modeling and translational regenerative medicine applications is facilitated by the availability of somatic cell-derived induced pluripotent stem cells (iPSCs). In that regard, the common marmoset monkey (Callithrix jacchus) has been identified as an ideal species for modeling age-related disorders, such as Parkinson's disease (PD), due to their shorter lifespan compared to larger nonhuman primates. While neuronal differentiation has been achieved from marmoset embryonic stem cells (Cj-ESCs) and iPSCs derived from fetal tissues, production of patterned midbrain floorplate (FP) neuroprogenitors (NPs) from adult marmoset fibroblast derived iPSCs have not been reported. The aim of this study was to fill this gap by producing a Cj-iPSC line from adult marmoset skin fibroblasts, generate a differentiation protocol to pattern FP-derived midbrain NPs, and characterize the expression of pluripotent, neural ectoderm, NP, and mature neuronal subtype genes throughout the differentiation process of both Cj-ESCs (Cj367) and Cj-iPSCs (M8). To reprogram the marmoset skin fibroblasts, skin punch biopsy tissue obtained from an adult marmoset was cut to smaller samples and explanted to 35 mm wells. Non-integrating pluripotency-inducing plasmids were then electroporated into the outgrown fibroblasts. To verify pluripotency of colonies selected by morphological criteria, RT-PCR was performed using primers for marmoset OCT4, SOX2, NANOG, KLF4, LIN28, and C-MYC genes. For ventral patterning, sonic hedgehog (SHH) was added at 500 ng/mL for the first 16 days, then reduced to 20 ng/mL until day 28. FP derivation was confirmed by a shift of expression from PAX6 to FOXA2. Anterior-posterior patterning was accomplished using CHIR99021 (0.4 μM). Colocalization of OTX2 and EN-1 confirmed midbrain identity. For neuronal differentiation, suspension neurospheres were transferred to adherent culture on day 28 and exposed to BDNF, GDNF, TGF-β3, cAMP, and Ascorbic Acid in neural basal media. Immunocytochemistry for nestin and βIII-tubulin confirmed NP and mature neural fates respectively. Our results demonstrate that the M8 line reprogrammed from adult marmoset fibroblasts is morphologically identical to the Cj367 ESC line, and pluripotent mRNA expression was similar between the two cell lines, each expressing all 6 endogenous genes mentioned above. We also observed that both M8 and Cj367 cell lines patterned to midbrain FP NPs under ventral-posterior patterning parameters and produced βIII-

tubulin positive neurons. We conclude that adult marmoset fibroblast-derived iPSCs can be patterned towards midbrain FP NPs.

Disclosures: S.C. Vermilyea: None. **S. Guthrie:** None. **M. Meyer:** None. **K. Braun:** None. **K. Smuga-Otto:** None. **S. Howden:** None. **J.A. Thomson:** None. **S. Zhang:** None. **T.G. Golos:** None. **M.E. Emborg:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.16/B42

Topic: A.03. Stem Cells

Support: NCTR/FDA E7417

Title: Using neural stem cells to evaluate the potential developmental neurotoxic effects of silver nanoparticles

Authors: *F. LIU[1], M. MAHMOOD[2], Y. XU[2], F. WATANABE[2], A. S. BIRIS[2], D. K. HANSEN[1], A. INSELMAN[1], D. CASCIANO[2], T. A. PATTERSON[1], M. G. PAULE[1], W. SLIKKER, Jr.[1], C. WANG[1];
[1]Natl. Ctr. For Toxicological Research/FDA, Jefferson, AR; [2]Ctr. for Integrative Nanotechnology Sci., Univ. of Arkansas at Little Rock, Little Rock, AR

Abstract: Silver nano-particles (Ag-NPs) are becoming increasingly prevalent in consumer products as antibacterial agents. The increased use of Ag NP-enhanced products will almost certainly increase environmental silver levels, resulting in increased exposures and the potential for increased adverse reactions including neurotoxic effects. In the present study, embryonic neural stem cells (NSCs) from human and rat fetuses (gestational day-16) were used to determine whether Ag-NPs are capable of causing developmental neurotoxicity. The NSCs were cultured in serum free medium supplemented with appropriate growth factors. On the eighth day *in vitro* (DIV 8), the cells were exposed to Ag-NPs at concentrations of 1, 5, 10, and 20 μg/ml for 24 h. The cultured cells then were characterized by NSC markers including nestin and SOX2 and a variety of assays were utilized to determine the effects of Ag-NPs on NSC proliferation and viability and the underlying mechanisms associated with these effects. The results indicate that mitochondrial viability (MTT metabolism) was substantially attenuated and LDH release was increased significantly in a dose-dependent manner. Ag-NPs-induced neurotoxicity was further confirmed by up-regulated Bax protein expression, an increased number of TUNEL-positively

stained cells, and elevated reactive oxygen species (ROS). NSC proliferation was also significantly decreased by Ag-NPs. Co-administration of acetyl-L-carnitine, an antioxidant agent, effectively blocked the adverse effects associated with Ag-NP exposure.

Disclosures: F. Liu: None. **M. Mahmood:** None. **Y. Xu:** None. **F. Watanabe:** None. **A.S. Biris:** None. **D.K. Hansen:** None. **A. Inselman:** None. **D. Casciano:** None. **T.A. Patterson:** None. **M.G. Paule:** None. **W. Slikker:** None. **C. Wang:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.17/B43

Topic: A.03. Stem Cells

Support: UMA, Campus Excel. Internat. Adalucia Tech

Norwegian Research Council (Grant 215086)

Funds Karolinska Institutet Sweden

Title: Positive biocompatibility of several graphene derivatives with dopaminergic cells at long term culture

Authors: N. RODRIGUEZ-LOSADA[1], R. WENDELBO[2], E. ARENAS[3], *J. A. AGUIRRE[1]; [1]Dept. of Physiology. Fac. of Med., Malaga, Spain; [2]Abalonyx AS, Oslo, Norway; [3]Dept. of Med. Biochem. and Biophysics, Karolinska Institutet, Stockholm, Sweden

Abstract: The emerging carbon nanomaterial graphene (G) and its oxidized derivative graphene oxide (GO) have recently gained considerable attention in biomedical applications such as cancer therapy or biosensors. It has for example been demonstrated that G has an efficient bioconjugation with common biomolecules and activates cell differentiation of neuronal stem cells (Li et al., 2013). This way, G could acts as a physical support or scaffold to promote differentiation and axonal sprouting of dopaminergic (DA) cells derived from neural stem cells. Since GO in its multilayer form and with multiple carboxylate and epoxy groups seems to show interesting biological properties (Yang et al., 2013) the aim of the present work has been to test different graphene derivatives searching for the best scaffold to be used in stem cell differentiation. For this purpose we have tested the cytotoxicity of GO and reduced GO, and specifically its biocompatibility with SN4741, a dopaminergic cell line derived from mouse substance nigra, measuring the effect on long term culture. The cells were cultured in Dulbecco's

modified Eagle's medium 10% FCS (Gibco) to about 80% confluence. Cells (1.000) were plated onto 96-well microliter plates with graphene using three chemically different types of GO as powders and films: 1) hydrophilic GO; 2) partially reduced GO (PRGO) which is hydrophobic and 3) fully reduced GO (FRGO), also hydrophobic, each of them in five concentrations: 1 mg/ml; 0.1 mg/ml; 0.05 mg/ml; 0.02 mg/ml and 0.01 mg/ml. Cells were cultured with GO and cell viability was determined after 24 hours, 1 week and 2 weeks using the MTT assay (Roche) and cytotoxicity was determined by the lactate dehydrogenase (LDH) assay (Roche). Our results show positive biocompatibility between the G-derivatives and SN4741 cells. We conclude that the use of our G-derivative scaffolds can enhance the morphological differentiation towards DA neurons (TH positive) providing microenvironments appropriate for neural differentiation and axon guidance. These findings suggest that biocompatible scaffolds can contribute to the future generation of successful clinical applications of G. Future experiments will examine whether G could offer a platform for neural stem cell and neural regeneration for neurological diseases such as PD. (Refs: Li N., Zhang Q, Gao S. et al., 2013, Nature/Sci Rep. 3:1604. doi: 10.1038/srep01604; Yan K., Li Y., Tan X., et al., 2013, Small., 9(9-10): 1492-1503) This work has been supported by the University of Malaga, Campus de Excelencia Internacional Andalucía Tech, Spain; The Norwegian Research Council (grant nº 215086) and funds from Karolinska Institutet, Stockholm, Sweden.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: A.03. Stem Cells

Support: HKRGC-General Research Fund 777810

NSFC/RGC-Joint Research Scheme N_HKU741/11

Innovation and Technology Fund 100/10

SK Yee Medical Research Fund

Title: Small molecule approach to direct differentiation of human induced pluripotent stem cells to sensory neurons

Authors: *D. K.-Y. SHUM[1], S. CAI[1], L. HAN[2], Y. S. CHAN[2];
[1]Dept. of Biochem., Fac Med, The Univ. of Hong Kong, Hong Kong, China; [2]Dept. of Physiol., Fac Med, The Univ. of Hong Kong, Hong Kong, China

Abstract: Strategies that exploit induced pluripotent stem cells (iPSCs) to derive neurons have relied on cocktails of cytokines and/or growth factors to bias cell signaling events in the course of fate choice. These are often costly and inefficient, involving multiple steps. In this study, we took an alternative approach and selected five small-molecule inhibitors of key signaling pathways to improve efficiency in derivation of sensory neurons from human iPSCs. Within 8 days of the differentiation protocol, iPSC-derived sensory neurons were achieved as denoted by marker expression, >80% being immuno-positive for Tuj1, NeuN and NF200, Islet1, peripherin and Brn3a but immuno-negative for neural progenitor markers (Pax6 and nestin) as well as neural crest cell markers (AP2, HNK1 and p75). Patch-clamp recordings on the derived neurons revealed healthy resting membrane potentials averaging -60 mV. Single action potentials were elicited in response to depolarizing step currents whereas multiple action potentials could be evoked with increasing intensity of the depolarizing current. Spiking was blocked by bath administration of tetrodotoxin. The derived cells therefore demonstrated electrophysiological properties characteristic of functional neurons. Neurite bundles that extended from the derived neurons were amenable to myelination in co-culture with rat Schwann cells, showing internodal segments that were immuno-positive for myelin-related proteins, P0, MBP and GALC. The phenotype of the iPSC-derived neurons was sustainable in Neurobasal medium supplemented with maintenance growth factors but without the small-molecule inhibitors. With this rapid and efficient induction protocol, we expect production of sensory neurons from human iPSCs on demand for developmental studies and disease modeling.

Disclosures: **D.K. Shum:** None. **S. Cai:** None. **L. Han:** None. **Y.S. Chan:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.19/B45

Topic: A.03. Stem Cells

Support: Harvard Stem Cell Institute

Title: Pre-clinical *in vitro* and *in vivo* characterization of xeno-free and foot-print-free iPSC-derived cell preparations for Parkinson's disease cell therapy

Authors: *T. M. OSBORN[1], P. J. HALLETT[1], M. MOORE[1], A. CASLER[1], D. DINESH[1], C. SKORIK[2], J. A. KORECKA[1], A. ASTRADSSON[1], J. SCHUMACHER[1], R. SPEALMAN[1], T. M. SCHLAEGER[2], O. ISACSON[1];
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Abstract: Parkinson's disease (PD) is a chronic progressive disorder with motor symptoms characterized by tremor, bradykinesia, rigidity and postural instability. Currently there are nearly one million diagnosed cases in the US. Clinical studies have shown that patients can gain improved motor function with transplantation of cell preparations derived from fetal ventral midbrain. However, fetal cell sources are too limited and require immunosuppression. Induced pluripotent stem cells (iPSCs) can be generated from affected PD patients or HLA-matched individuals and differentiated to midbrain dopaminergic cells, providing opportunities for low-rejection risk or autologous transplantations. We have recently shown that dopaminergic neurons derived from iPSCs from an MPTP-lesioned cynomolgus monkey survived two years after autologous transplantation, re-innervated the host putamen and provided improved motor function (Hallett et al. Cell Stem Cell. 2015 Mar 5;16(3):269-74). We did not observe any graft-derived proliferating cells two years after transplantation, which is encouraging from a clinical standpoint. We are now in pre-clinical experiments improving our cell differentiation and transplantation paradigms in order to outline requirements and conditions for potential clinical trials. Using a xeno-free differentiation protocol (modified from Cooper et al., 2010, Mol Cell Neurosci;45(3):258-66) and feeder-free and foot-print-free human iPSC lines derived using episomal reprogramming technology, we are defining positive and negative cell-marker expression criteria of cell-preparations and cell-sorting requirements. We are also determining cell freezing and thawing strategies and pre-transplantation cell-viability criteria. For safety purposes we are preparing for scale-up and cell-dosing studies. Functional recovery and graft survival is studied in xeno-grafted rodents. These data and experiments are IND enabling efforts to establish future clinical trials.

Disclosures: **T.M. Osborn:** None. **P.J. Hallett:** None. **M. Moore:** None. **A. Casler:** None. **D. Dinesh:** None. **C. Skorik:** None. **J.A. Korecka:** None. **A. Astradsson:** None. **J. Schumacher:** None. **R. Spealman:** None. **T.M. Schlaeger:** None. **O. Isacson:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.20/B46

Topic: A.03. Stem Cells

Support: nih grant

Title: HESX1 regulates neural induction from human embryonic stem cells

Authors: ***C. T.-L. HUANG**[1,2], J. LU[1], L. FOWLER[1], Y. CHEN[1], J. CAO[1], S.-C. ZHANG[1,2,3]; [1]Waisman Center/University of Wiscosin, Madison, Madison, WI; [2]Dept. of Neurosci., [3]Dept. of Neurol., Univ. of Wiscosin, Madison,, Madison, WI

Abstract: The precise coordination of signaling pathways and transcriptional regulation is required for neural development. However, how the neural induction process is coordinated remains unknown. Here we use the *in vitro* neural induction model from human embryonic stem cells (hESCs) and show that HESX1, a homeodomain transcription factor, is transiently expressed right before a neural determinant marker, PAX6, is expressed. Knockout (KO) of HESX1 does not affect the self-renewal and pluripotency of hESCs. It, however, results in early neural induction, indicated by the earlier and faster downregulation of pluripotent markers, such as OCT4 and NANOG, and the accelerated upregulation of neural induction markers, such as PAX6 and ZEB2. In addition, the structure of neural tissue in the teratoma from the HESX1-KO hESCs is disorganized. Interestingly, non-physiological overexpression of HESX1 under the HESX1 KO background promotes neural induction. Our findings suggest that HESX1 plays a role as a gate-keeper in human neural induction.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

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Topic: A.03. Stem Cells

Title: Functional properties of cortical neurons derived from human induced pluripotent stem cells maintained in a single-cell and feeder-free culture

Authors: ***T. ARAKI**[1], T. ONO[2], K. TESHIMA[3], T. SHIRAKAWA[2], H. AOYAMA[2], Y. KATO[2], T. YAMASHITA[2], A. DOI[2], S. KOBAYASHI[2], Y. SUZUKI[2], N. SATO[2], Y. KOGUCHI[2], M. SAKURAI[1];

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Abstract: Pluripotent stem cells (PSCs) including embryonic stem cells and induced pluripotent stem cells (iPSCs) proliferate infinitely and differentiate into multiple cell types. Neuronal progenitor cells (NPCs) and functional mature neurons derived from human PSCs can be useful in regenerative medicine and drug discovery. However, human PSCs under a conventional culture, growing as colonies on feeder cells, often differentiate into undesired cells that interrupt reproducible experiments and the large-scale preparation of functional neurons. Here we report the robust differentiation into highly pure NPCs and neurons from human iPSCs maintained in a single-cell and feeder-free (SFF) culture using an original chemically defined medium containing bFGF and Activin, by which human PSCs serve as a practical cell source. Differentiated cells formed rosette-like structures and expressed neuronal markers such as β3-tubulin and microtubule-associated protein 2. In addition, we have established the techniques for cryopreservation of the NPCs, which enable highly reproducible functional analysis and drug screening practices. To characterize functional properties of the neurons, we performed whole-cell patch-clamp recording at 1-4 weeks after re-plating. In current clamp-mode, depolarizing current injection revealed the generation of action potentials. In voltage clamp-mode, depolarizing voltage steps from a holding potential of -60 mV elicited fast inward Na^+ currents that were blocked by application of tetrodotoxin (TTX). Moreover, application of both glutamate and gamma-aminobutyric acid (GABA) elicited inward currents at a holding potential of -60 mV. The electrophysiological analysis revealed essential functional properties such as TTX-sensitive voltage-dependent Na^+ currents, glutamate- and GABA-induced currents in the neurons. Thus, the functional human iPSC-derived neurons are expected to be a powerful tool for disease investigation and drug development. Furthermore, it is suggested that the SFF culture for human iPSCs is an outstanding versatile platform to prepare several functional cells including neurons.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

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Topic: A.03. Stem Cells

Support: Swedish Research Council

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Alzheimerfonden and the Parkinson Research Foundation

Title: Expression of neurogranin during differentiation of hiPS cells to cortical neurons

Authors: *F. H. NAZIR[1], L. AGHOLME[1], K.-P. HUANG[2], E. PORTELIUS[3], H. KVARTSBERG[3], H. WELLINGTON[4], K. BLENNOW[3], H. ZETTERBERG[3,4], P. BERGSTRÖM[1];
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Abstract: Background Neurogranin (Ng), a post-synaptic, brain specific protein kinase C substrate, is expressed in neuronal cell bodies and dendrites. Ng has a role in synaptic plasticity, long term potentiation, spatial memory and learning. Much research has been done on the expression of Ng during development in rodent models, while little is still known about the expression of Ng in the human brain. Due to differences in the development and maturation of the human brain compared with the rodent brain, rodent brain models are inadequate to address some specific scientific questions. One example is expression of Ng during cortical development. Therefore, cells of human origin were used here to study the expression of Ng during cortical development. Methods Human induced pluripotent stem cells (hiPS cells) of chondrocyte and fibroblast origin were differentiated towards cortical neurons following Shi et al., 2012 protocol. Cell conditioned media was collected continuously during the 125 days long differentiation and secreted levels of Ng were measured in the media using ELISA and immunoprecipitation-mass spectrometry (IP-MS). Expression of Ng was assessed with immunocytochemistry (ICC) at different days of differentiation. Results ICC results showed that cellular expression of Ng appears at approximately 40 to 50 days of differentiation from hiPS cells towards cortical neurons. During differentiation Ng was observed to translocate from nuclei to neurites. ELISA results indicated that Ng was secreted to the cell conditioned media throughout differentiation. Further, it was possible to detect Ng peptide (53-78) in the cell conditioned media via IP-MS. Conclusions To the best of our knowledge, cellular expression of Ng from cells of human origin was illustrated for the first time during differentiation. Furthermore, detection of Ng peptide (58-78) and secretion of Ng in cell conditioned media throughout differentiation are also novel findings in this study. Taken together, this study provides a promising platform for elucidating the role of Ng in human cortical development.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 665.23/B49

Topic: A.03. Stem Cells

Title: Treatment with gamma–secretase inhibitor for neural stem/progenitor cells derived from tumorigenic human induced pluripotent stem cells

Authors: ***T. OKUBO**[1,2], A. IWANAMI[2], J. KOHYAMA[3], G. ITAKURA[2], M. MASTUMOTO[2], M. NAKAMURA[2], H. OKANO[3];
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Abstract: [Introduction] Recently we reported that transplantation of neural stem/progenitor cells derived from human induced pluripotent stem cells (hiPS-NSC) promoted functional recovery in animal models of spinal cord injury (SCI). However, some cell line of hiPS-NSC occurred deterioration of motor function due to tumor formation after transplantation. Since Notch signaling controls the induction of definitive neural stem cells, its inhibition with Gamma-secretase inhibitor (GSI) may induce hiPS-NSC to differentiate into a more mature state with limited proliferation. Here we present the effects of GSI on proliferation and differentiation of hiPS-NSC. [Method] hiPS-NSC (253G1), a potentially tumorigenic cell line (Nori et al, Stem Cell Reports 2015), were cultured with GSI for 1 day in GSI-1d group, 4 days in GSI-4d group, and without GSI in no GSI treatment group. Then the characterizations of these cells were evaluated by immunocytochemistry and the neuronal maturation was also assessed by micro electrode array (MEA). Cell cycle/apoptosis analysis was performed using flow cytometry, and the gene expression related to pluripotency, self-renewal and neural differentiation were analyzed using RT-PCRs. All of these data were statistically analyzed and compared among three groups. [Result] In the GSI-4d group, the number of cells significantly decreased compared to the other groups. In the no GSI treatment group, the sizes of spheres were significantly larger than those of both GSI groups. Compared to the no GSI treatment group, the percentage of G0/G1 phase significantly increased, and the percentage of S phase significantly decreased in both the GSI groups, although there was no significant difference in AnnexinV/7-AAD apoptosis assay. The percentages of Ki67-and Nestin-positive cells also significantly decreased compared

with no GSI treatment group, whereas the percentage of Tuj-1-positive cells significantly increased. In both GSI groups, neuronal maturation was significantly enhanced compared to the no GSI treatment group. RT-PCRs showed that the expression of Notch signaling target genes, such as HES5, Notch1 were almost abolished by GSI, indicating that Notch signaling was efficiently inhibited. Gene expression of the mature neuronal markers, such as Neurog2, ASCL1, NeuroD1, but not glial markers, such as GFAP, Olig2, were up-regulated in accordance with the down-regulation of gene expression of pluripotency/self renewal markers, such as Nanog, Lin28, Oct3/4, Nestin. [Conclusion] In conclusions, pretreating with GSI promoted neuronal differentiation and maturation, inhibited glial differentiation of hiPS-NSC *in vitro*.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

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Topic: A.03. Stem Cells

Support: the National Natural Science Foundation of China (81170885)

Title: Study on the cell transplantation and differeniation of retinal progenitor derived from human embroynic stem cells

Authors: ***S. WANG**, X. WANG, K. XIONG, D. GU, G. ZHOU;
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Abstract: Human embryonic stem cells (hESCs) have been reported to serve as an excellent source for obtaining various specialized cell types and could be used in cell replacement therapy. Here we demonstrate the potential of hESCs-GFP to differentiation into retinal progenitor by using our novel differentiation system. The neural optic cup-like structures can be formed within 4-5 weeks. Most of the cells were pax6 and math5 positive, and $Math5^{+}$/$Brn3b^{+}$ ganglion cell progenitor were deceted in the hESC-derived optic cup-like structures at 8 weeks. When these cells were transplanted into the vitreous cavity of mice that were damaged by N-methyl-D-aspartate (NMDA) before, at 30 days past transplantation, the transplanted cells could survive and integrate into the host retina, located in the ganglion cell layer. At 35 days, some of them were Brn3a positive. These findings suggests that retinal progenitor cells derived from hESCs can differentiate into retinal ganglion cells under the host retinal microenvironment. Our results

provide insights that hESCs can serve an excellent renewable source for generating retinal progenitor that can be used to treat neurodegenerative disease like glaucoma.

Disclosures: **S. Wang:** None. **X. Wang:** None. **K. Xiong:** None. **D. Gu:** None. **G. Zhou:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

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CART Pilot Grant

CIRM postdoctoral fellowship

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NIH Grant HD059967

Title: Reproducible generation of functionally active networks of GABAergic and glutamatergic neurons through directed differentiation of human iPSCs

Authors: ***Y. XIE**[1], R. J. SCHUTTE[1], N. N. NG[1], A. T. PHAM[1], S. S. SCHUTTE[1], M. G. BANUELOS[2], A. E. STOVER[2], K. ESS[3], A. L. GEORGE, Jr.[4], M. A. SMITH[1], P. H. SCHWARTZ[2], D. K. O'DOWD[1];
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Abstract: Genetic epilepsy with febrile seizures plus (GEFS+) is an autosomal dominant disorder associated with mutations in *SCN1A,* encoding the $Na_v1.1$ voltage-gated sodium channel. Our lab has used a *Drosophila* knock-in model to explore the cellular mechanisms contributing to hyperthermia-induced seizures characteristic of GEFS+ patients. These data suggest that the K1270T mutation causes hyperthermia-induced seizures through a conditional gain-of-function alteration in sodium channels that reduces excitability of GABAergic neurons (Sun *et al.*, 2012). To determine whether this mutation causes similar changes in sodium channels and excitability in human neurons, we generated induced pluripotent stem cell (iPSC)

lines from three siblings, two with the GEFS+ K1270T mutation and one without (Control). We used a recently published strategy for directed differentiation of iPSCs into medial ganglionic eminence (MGE) progenitors that had been shown to enrich cultures for GABAergic neurons (Liu *et al.*, 2013). Three weeks after plating MGE progenitors onto mouse astrocyte feeder layers, approximately 30% of the MGE derived neurons arising from Control iPSCs were GABAergic and nearly all recorded cells were capable of firing action potentials. Single action potentials were observed as early as ten days after neuronal differentiation was begun. Functional maturation continued steadily and by five weeks in culture the majority of neurons examined fired spontaneous action potentials and had GABAergic and/or glutamatergic synaptic inputs. The time course and degree of functional differentiation was similar from plating to plating, allowing quantitative analysis of currents, firing properties and synaptic transmission. Therefore comparison of cultures prepared from Control and GEFS+ iPSCs will allow us to identify alterations in sodium currents, firing properties and synaptic function associated with the K1270T mutation in human neurons. This differentiation protocol will also be useful in determining how other genetically induced neurological disorders affect neuronal maturation and function, facilitating development and testing of novel therapies.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

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Topic: A.03. Stem Cells

Support: NIH Grant EY024940

BrightFocus G2012027

Title: *In vitro* modeling of early retinogenesis with human pluripotent stem cells

Authors: ***A. SRIDHAR**[1], S. K. OHLEMACHER[1], J. S. MEYER[1,2];
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Abstract: Human pluripotent stem cells (hPSCs) provide a unique ability to study some of the earliest events of human development, particularly some of the earliest events in human retinogenesis such as the establishment of a definitive retinal fate from a more primitive neural progenitor source. In this role, hPSCs may provide an *in vitro* model for understanding the complex interplay of transcription factors involved in the acquisition of a retinal fate from an unspecified pluripotent cell population. hPSCs were differentiated as previously described and samples were collected every two days, starting from the undifferentiated state through when cells acquired either retinal or non-retinal forebrain identities. Immunocytochemistry and qRT-PCR approaches were undertaken to identify candidate transcription involved in retinal fate establishment. Candidate transcription factors were identified underlying the establishment of a retinal fate apart from other neural lineages. Neural transcription factors including PAX6 and OTX2 were expressed early while retinal-associated transcription factors such as SIX6 were expressed at slightly later timepoints. Upon establishment of an anterior neural identity, the expression pattern of the RAX transcription factor became more restricted to subpopulations of cells, presumably indicative of the emergence of retinal population apart from related forebrain populations from the same primitive anterior neural population. Gene overexpression and knockdown experiments investigated the mechanism of action of these candidate transcription factors. Furthermore, epigenetic analysis demonstrated that DNA methylation could potentially account for differential gene expression in the establishment of retinal phenotypes apart from alternate neural lineages. Preliminary results begin to elucidate the complex interplay of transcription factors involved in the specification of a retinal fate from differentiating hPSCs. Overall, these results will help to better establish hPSCs as a valuable *in vitro* system with which to study some of the earliest events of human retinogenesis.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

Location: Hall A

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Topic: A.03. Stem Cells

Support: Core Program for Disease Modeling using iPS Cells from JST

Scientific Research (C) frrom JSPS (24570242)

Ministrary of Health, Labour and Welfare (24-9)

Title: Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells

Authors: *K. MUGURUMA[1], H. KAWAKAMI[2], K. HASHIMOTO[2], Y. SASAI[3];
[1]Cell Asymmetry RIKEN CDB, Kobe, Japan; [2]Hiroshima Univ., Hiroshima, Japan; [3]RIKEN CDB, Kobe, Japan

Abstract: The cerebellum (CB) has been considered as a major component of the motor system. It is well known that the damage in the CB leads impairments in motor and postural control. In human, the CB develops over a long period of time, from the early embryonic days to the first postnatal year. Such protracted development could be the source of vulnerability and the cause of broad spectrum of developmental disorders. Cellular and molecular studies on the cerebellar development have been done mostly in model animas. However, it is not known whether the mechanisms revealed in model animals are simply applicable or not. Thus the studies in human have been long awaited for elucidation of functions of the human CB. The CB is a highly ordered brain structure with several well-defined types of cells. The initial phase of cerebellar development is the formation of the isthmic organizer. Under its inductive influence, the cerebellar anlage arises in the dorsal region of rhombomere 1 (r1). Cerebellar cells are generated in the two distinct germinal zones in the r1. One is the ventricular zone of the cerebellar plate (CP), which expresses Ptf1a. The Ptf1a progenitors produce GABAergic neurons of the cerebellar cortex (Purkinje cells and interneurons) and of the deep cerebellar nuclei (DCN). The other one is the upper rhombic lip (RL), which expressed Atoh1. The Atoh1 progenitors generate cerebellar glutamatergic neurons, including granule cells (GC) and large DCN projection neurons. We previously reported that cerebellar neurons could be generated from mouse embryonic stem cells (mESCs). mESCs have the potential to form isthmic organizer tissue in response to FGF2 and Insulin. Here, we apply the self-formation principle to human ESC/iPSC culture for the generation of human cerebellar tissues *in vitro*. Using modified culture condition, we show *in vitro* production of major cerebellar types such as Purkinje cells and GCs. In the course of optimizing 3D culture we identified two factors, FGF19 and SDF1 that promotes self-formation of ordered CP-like tissues in distinct manners. FGF19 promotes spontaneous generation of dorsoventrally polarized neural tube-like structures at the level of the CB. Furthermore, addition of SDF1 and FGF19 promotes the generation of a continuous CP neuroepithelium with RL-like structure at one end a three-layer cytoarchitecture similar to the embryonic CB. Thus, human pluripotent stem cell-derived cerebellar progenitors exhibit substantial self-organizing potential for generating a polarized structure reminiscent of the early human CB at the first trimester.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

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Topic: A.03. Stem Cells

Title: Improved differentiation of mouse embryonic stem cells into Purkinje Neurons

Authors: *C. J. ALEXANDER, J. A. HAMMER, III;
Lab. of Cell Biol., NHLBI, NIH, Bethesda, MD

Abstract: The use of embryonic mixed primary cerebellar cultures has been invaluable for dissecting detailed structure: function studies in Purkinje Neurons (PNs), however this technique is technically challenging and can yield few cells. Recently, mouse embryonic stem cells (mESCs) have been successfully differentiated into PNs, however these methods are equally as challenging as primary cultures. The focus of this study was to determine if we can simplify differentiation of mESCs into PNs. Using specific extrinsic factors, we have successfully differentiated mESCs into PNs without the requirement for a postnatal feeder-layer. We have characterized the morphology of mESC derived PNs and show that they are indistinguishable from PNs grown in primary culture with similar gross morphology, spine length and spine density. Further to this we have demonstrated that mESC derived PNs express Calbindin D28K, IP3R1, PLCb4 and GRID2, all of which are PN-specific markers. Finally, using collagen, poly-l-lysine and gelatin as the extra cellular matrix has allowed us to grow mESC derived PNs in monolayers, which will be critical for live cell imaging. Future directions will be to determine if we can express exogenous DNA specifically in the mESC derived PNs using the PCP2/L7 PN-specific promoter. If this is possible, we will be able to gene edit stem cells using CRISPR and compliment with exogenous DNA. Together, this will allow for a scalable, high throughput approach that will be important in dissecting specific molecular mechanisms in PNs, especially in currently unobtainable genotypes.

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Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

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Topic: A.03. Stem Cells

Support: CIRM grant Tr4-06648

Title: Molecular characterization of human stem cells differentiated into transplantable retinal sheets via 3d neurosphere retinogenesis

Authors: B. T. MCLELLAND[1], A. MATHUR[1], C. TSE[1], T. ESTRADA-HERNANDEZ[2], S. KAYSER[2], G. MISTOR[2], H. S. KEIRSTEAD[2], *M. J. SEILER[1];
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Abstract: Age-related macular degeneration and other retinal disorders affect millions of people worldwide. Current treatments can delay the degradation, but few can restore function and visual acuity. The challenge is how best to create and introduce fresh, healthy tissue to replace damaged host retinal cells. We used human embryonic stem cells (hESC) to produce transplantable sheets of retinal and retinal pigmented epithelium (RPE) and are now testing the use of induced pluripotent stem cells (iPSC). It is hoped that the new iPSC derived tissue will create fresh photoreceptors for the host, generate new synaptic connections for phototransduction, and a new RPE monolayer critical for photoreceptor maintenance. Human stem cells were differentiated into retinal tissue by creating 3D neurospheres, following a protocol modeled after Zhong et al. 2014 (Nature Communications 5:4047) which results in laminated 3D structures (neurospheres). Quantitative polymerase chain reaction (qPCR) analysis of genes characteristic for neuronal (Pax6) and retinal development (Chx10, Rax, CRALBP, CRX, recoverin), and RPE (MITF) was performed on retinal neurospheres between d27 and d73 of differentiation and human stem cell-derived RPE monolayers. For comparison, d84 and d110 human fetal retina (HFR) and RPE were also analyzed and used as control tissues. In parallel, immunofluorescence (IF) experiments on fixed differentiated neurospheres looked for the expression of important neuro-retinal proteins found in the human eye (e.g. Rax, CRX, Recoverin, CRALBP, MITF). Immunohistochemical H+E staining confirmed the developing lamination and retinal maturation of the neurospheres at progressive time points. Taken together, the data demonstrates a neurosphere expression pattern similar to that seen in the developing human fetal eye with early commitment progenitor markers like PAX6 and CHX10 coming up first during the culture time course and more mature photoreceptor and RPE proteins (i.e. recoverin and CRALBP) coming up late. The 3D neurospheres are clearly undergoing retinogenesis and the use of this system to produce transplantable tissue is promising. Transplantation experiments into a nude S334ter-line 3 retinal degenerate rat strain are ongoing.

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Employment/Salary (full or part-time):; 3)California Stem Cell, Inc., Irvine CA (since acquired by NeoStem, Inc.). **H.S. Keirstead:** A. Employment/Salary (full or part-time):; 3)California Stem Cell, Inc., Irvine CA (since acquired by NeoStem, Inc.). **M.J. Seiler:** None.

Poster

665. Induced Pluripotent Stem Cells: Neural Differentiation

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Support: Grant-in-aid for Young Scientists (B) from Japan Society for the Promotion of Science (JSPS)

Title: Visualization of photoreceptors derived from human iPSC by using CRISPR/Cas9 system

Authors: *K. HOMMA, M. KANEDA;
Nippon Med. Sch., Tokyo, Japan

Abstract: Genome engineering tools, such as ZFN, TALEN, and CRISPR/Cas9 systems enable us to efficiently insert fluorescent reporter gene(s) into the genome. The cell lineage specific reporter gene knock-in induced-pluripotent stem cell (iPSC) lines are used to evaluate the efficiency of differentiation/maturation in physiological experiment or live cell imaging. Here, we introduced CRISPR/Cas9 system to insert fluorescent protein gene into the C-terminal of photoreceptor specific transcriptional factor. Photoreceptor specific genes were linked with fluorescent protein gene by the gene of 2A-peptide, which was to be cleaved off after the translation. Another target site is the adeno-associated virus integration site 1 (AAVS1), which is enclosed by insulators, thus is thought as “safe harbor” for gene insertion. The guide-RNA expression vectors, the Cas9 expression vectors, and the donor vector, which contains puromycin or blasticidine resistance gene, were electroporated into human iPSCs (454E2 line from RIKEN BRC). Electropolated human iPSCs were plated on Matrigel-coated 6 well plates. On day 2 after the electroporation, antibiotic, such as puromycin or blasticidine was added to the culture medium. After the selection culture, around the day 10, possible single colonies were picked and amplified on culture plate. Genomic PCR showed that the donor gene was inserted into target sites in 19 lines / 20 lines (95.0 %). Also, genomic sequencing confirmed correct gene insertion in these hiPSC lines. After the 3D retinal differentiation culture, retinal laminar formation was observed and some cells showed fluorescence in expected layer. These photoreceptor-specific reporter gene knock-in human iPSC lines are useful for identifying the developmental stage at which derived photoreceptors were applicable to retinal cell-based therapy.

Disclosures: **K. Homma:** None. **M. Kaneda:** None.

Poster

666. Synapse Refinement

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 666.01/B57

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

Support: NINDS (NS065048)

Title: Skilled grasping requires non-apoptotic Bax/Bak-mediated corticospinal circuit refinement

Authors: ***Z. GU**[1], N. SARRAD[2], M. L. BACCEI[3], J. LI[3], M. UENO[1], M. LIANG[1], J. H. MARTIN[2], Y. YOSHIDA[1];
[1]Div. of Developmental Biol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Dept. of Physiology, Pharmacol. and Neurosci., City Col. of the City Univ. of New York, New York, NY; [3]Pain Res. Center, Dept. of Anesthesiol., Univ. of Cincinnati Med. Ctr., Cincinnati, OH

Abstract: During mammalian development including humans, early postnatal animals are only capable of performing motor behaviors that are critical for survival, whereas fine motor skills are acquired later during postnatal developmentrefs. Therefore, reorganization of motor circuits, including corticospinal (CS) circuits, for skilled movements has been postulated to occur during postnatal development in an activity-dependent manner. However, it remains unknown how CS (or any other motor) circuits underlying skilled movements are refined during development to control appropriate patterns of muscle activation, and how neuronal activity controls the CS circuit refinement. Here we explored connectivity between CS neurons and functionally related muscle pairs in juvenile and adult mice by injecting two color variants of retrograde, trans-synaptic pseudorabies viruses (PRVs) to various functionally-related (synergistic or antagonistic) muscle pairs. We find that relatively distinct populations of CS neurons form circuits with antagonistic muscle pairs in juvenile mice, whereas similar populations do in adults, suggesting that antagonistic CS circuits undergo synaptic refinement during development. In contrast, we do not find synaptic refinement of synergistic muscle pairs. The circuit rearrangement of antagonistic muscle pairs does not occur in the absence of the activity-dependent, non-apoptotic Bax/Bak-caspase signaling pathway. Moreover, recording muscle activity in response to cortical stimulation reveals aberrant co-activation of antagonistic muscle pairs in adult Bax/Bak double mutant mice, likely due to defects in synaptic refinement of CS circuits. Finally these mutant mice show deficits in skilled grasping without affecting reaching behaviors. Our findings therefore reveal that acquisition of proper flexor and extensor activation during skilled

movements requires Bax/Bak-mediated synaptic refinement of antagonistic CS circuits during development. Since up to 6% of all children suffer from developmental motor disabilities affecting motor skill movements, these studies provide the potential mechanisms underlying skilled movement disorders related to CS circuits.

Disclosures: **Z. Gu:** None. **N. Sarrad:** None. **M.L. Baccei:** None. **J. Li:** None. **M. Ueno:** None. **M. Liang:** None. **J.H. Martin:** None. **Y. Yoshida:** None.

Poster

666. Synapse Refinement

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 666.02/B58

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

Support: NIH Grant EY019694

NIH Grant T32

Title: Activity-dependent synapse refinement requires the cytoskeleton regulator CRMP

Authors: ***S. CASPER**, T. HERMAN;
Biol., Univ. of Oregon, Eugene, OR

Abstract: Neuronal activity is a critical regulator of synapse specificity during development. We are using *Drosophila* R7 photoreceptor neurons to understand how activity prevents the formation of ectopic synapses. Wild-type R7s in the retina extend axons to a specific layer in the brain and assemble synapses within their terminal boutons. We have found that R7s lacking voltage-gated calcium channels (VGCCs) extend their axons normally and form terminal boutons in the correct layer, but later these boutons sprout thin projections that extend deeper into the brain and contain presynaptic markers. This defect resembles that caused by loss of VGCCs from vertebrate photoreceptors. We found that disrupting the calcium-dependent kinase CaMKII in R7s causes an identical defect, suggesting that prevention of these ectopic projections requires the influx of calcium. Although the projections form around the time that R7s are able to detect light, we found that animals reared in the dark have wild-type R7s, indicating that the VGCCs prevent ectopic projections by opening in response to spontaneous voltage changes and not in response to light. After establishing this model of activity-dependent synapse refinement, we performed an EMS screen to identify the genes that act downstream of VGCCs. Among others, we identified Collapsin Response Mediator Protein (CRMP), a cytosolic phosphoprotein

that has previously been shown to regulate axon growth via its effects on microtubules. *CRMP* mutants are viable and have normal locomotory activity, yet 30% of *CRMP* mutant R7 boutons sprout ectopic projections that phenocopy loss of VGCCs. *CRMP* is well-studied in vertebrates and *C. elegans*, yet nothing is known about its role in presynaptic development. We generated individual *CRMP* mutant R7s and found that they initially assemble synapses in the correct locations and only later extend ectopic projections. The conserved RhoGEF Trio has previously been shown to mediate presynaptic development in both R7s and motor neurons. We have found that loss of *trio* also causes R7 ectopic projections and that *CRMP* is partially rescued by over-expression of Trio, indicating that *CRMP* functions either upstream of or in parallel to the Trio pathway. We are currently testing a model in which CRMP acts downstream of VGCCs and CaMKII to regulate microtubule assembly, thereby localizing Trio and preventing the formation of ectopic projections.

Disclosures: S. Casper: None. **T. Herman:** None.

Poster

666. Synapse Refinement

Location: Hall A

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Program#/Poster#: 666.03/B59

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

Support: Swiss National Science Foundation Grant 31003A-130625

ERA-NET NEURON II CIPRESS (Academy of Finland)

Jane and Aatos Erkko Foundation

Title: KCC2-mediated Cl- extrusion prevents propofol-induced dendritic spine loss

Authors: *M. PUSKARJOV[1], H. FIUMELLI[2], A. BRINER[3,4], T. BODOGAN[3,4], K. DEMETER[3,5], C.-M. LACOH[3], K. KAILA[1], L. VUTSKITS[3,4];
[1]Univ. of Helsinki, Helsinki, Finland; [2]King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia; [3]Univ. of Geneva Med. Sch., Geneva, Switzerland; [4]Univ. Hosp. of Geneva, Geneva, Switzerland; [5]Inst. of Exptl. Med. of the Hungarian Acad. of Sci., Budapest, Hungary

Abstract: The long-term impact of early life anesthesia exposure on neurocognitive function is a potential public health issue. Administration of general anesthetics in the early postnatal period can lead to developmental stage-dependent life-long changes in synapse number in the central nervous system. The present study aimed to test the hypothesis that the effect of general

anesthetics on synaptogenesis depends upon the efficacy of GABAA receptor-mediated inhibition controlled by the developmental up-regulation of the potassium-chloride cotransporter KCC2. *In utero* electroporation of KCC2 or the inward-rectifier potassium channel Kir2.1 in timed-pregnant rats was used to prematurely increase the efficacy of GABAergic inhibition or to reduce neuronal excitability in layer 2/3 pyramidal neurons in the immature rat somatosensory cortex. The effects of these genetic manipulations on propofol anesthesia-induced changes in dendritic spine densities were evaluated using iontophoretic injection of Lucifer Yellow. We found a robust correlation between the developmental up-regulation of KCC2-mediated Cl- extrusion and the age-dependent effects of propofol on dendritic spines of pyramidal neurons. Early overexpression of KCC2 but not of its transport-deficient N-terminal deletion construct, KCC2-ΔNTD, completely prevented propofol-induced dendritic spine loss. Overexpression of Kir2.1, which dampens neuronal excitability, also protected against the impact of propofol on dendritic spines. These data demonstrate that the KCC2-dependent developmental increase in the efficacy of GABAA receptor-mediated inhibition is a major determinant of the age-dependent actions of propofol on dendritic spinogenesis in the developing cerebral cortex.

Disclosures: **M. Puskarjov:** None. **H. Fiumelli:** None. **A. Briner:** None. **T. Bodogan:** None. **K. Demeter:** None. **C. Lacoh:** None. **K. Kaila:** None. **L. Vutskits:** None.

Poster

666. Synapse Refinement

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Program#/Poster#: 666.04/B60

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

Support: Major State Basic Research Program of China (2011CB504400, 2013CB945600, 2015CB755600, 2015AA020515)

National Natural Science Foundation of China (31471022, 81221003, 91232000, 31490590)

Fundamental Research Funds for the Central Universities (2014FZA7007)

Title: Astrocyte-derived ATP mediates synapse eliminaton in the thalamus

Authors: ***J. YANG**, H. YANG, D. ZHOU, X. LI, L. QIN, H. LOU, S. DUAN, H. WANG; Dept. of Neurobio., Zhejiang, China

Abstract: The selective elimination of unwanted synapses is a key mechanism for the precise formation of neuronal circuits during development, but the underlying mechanisms remain unclear. Using mice deficient in the inositol 1,4,5-trisphosphate receptor type 2 (Itpr2−/−) to specifically disturb intracellular Ca2+ signaling in astrocytes, we showed that developmental elimination of the ventral posteromedial nucleus (VPm) relay synapse was impaired. Interestingly, intraperitoneal injection of ATP, but not adenosine, rescued the deficit in synapse elimination in Itpr2−/− mice. We next found that ATP activated P2Y1 receptors to mediate long-term depression and thus may serve as a "punishment" signal for synapse elimination. This hypothesis was further supported by the finding that developmental synapse elimination was also impaired in P2ry1−/− mice and was not rescued by ATP. Our results have uncovered a novel mechanism suggesting that astrocytes release ATP in an IP3R2-dependent manner to regulate synapse elimination by activating P2Y1 receptors.

Disclosures: **J. Yang:** None. **H. Yang:** None. **D. Zhou:** None. **X. Li:** None. **L. Qin:** None. **H. Lou:** None. **S. Duan:** None. **H. Wang:** None.

Poster

666. Synapse Refinement

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Program#/Poster#: 666.05/B61

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

Support: Canadian Institutes for Health Research Operating Grant MOP-14137

Canadian Foundation of Innovation (equipment grant)

Fonds Recherches Quebec-Santé (FRQ-S, infrastructure grant) to the Groupe de recherche sur le Système Nerveux Central

Title: Glial cells control synaptic plasticity of competing nerve terminals and alter synaptic connectivity at mammalian neuromuscular junctions

Authors: *H. DARABID[1,2], R. ROBITAILLE[1,2];
[1]Neurosci., Univ. de Montreal, Montreal, QC, Canada; [2]Groupe de recherche sur le systeme nerveux central, Univ. de Montreal, Montreal, QC, Canada

Abstract: The precise wiring of synaptic connections requires the elimination of supernumerary inputs competing for innervation of the same target cell. At the neuromuscular junction (NMJ), this competition depends on the synaptic efficacy of competing terminals which strengthens one

input whilst others are weakened and eventually eliminated. Little is known about the synaptic activity and plasticity of these competing terminals. Moreover, the role of glial cells during synaptic competition remains ill-defined despite their known importance in the modulation of synaptic efficacy and plasticity at adult NMJs. Therefore, the goal of this work was to study the regulation by perisynaptic Schwann cells (PSCs), the glial cells at NMJs, of synaptic plasticity of nerve terminals during synaptic competition and their influence on synaptic elimination. We performed intracellular recordings from dually innervated P7-8 mouse Soleus muscle fibres to assess synaptic activity and we monitored PSC activity using confocal Ca^{2+} imaging. PSCs were loaded with the Ca^{2+} indicator Fluo-4 by single cell electroporation. We observed a tight relationship between the size of PSC's Ca^{2+} responses and the synaptic strength of each input (i.e. weak input generated smaller Ca^{2+} responses than the strong one). Moreover, at the same NMJ, the strong input showed a long-lasting potentiation of neurotransmission while the weak one displayed only a small transient potentiation. This differential plasticity of competing terminals depends on PSCs Ca^{2+} signalling since inhibiting PSCs Ca^{2+} activity, by photoactivation of Diazo-2 (photoactivable BAPTA loaded into PSCs by single cell electroporation), blocked PSCs Ca^{2+}-responses and resulted in the prevention of synaptic plasticity. The controlled raise of Ca^{2+} in PSCs using the caged-compound NP-EGTA was sufficient to induce synaptic plasticity. Furthermore, bath application of the purinergic P2Y1 receptors antagonist MRS2179 blocked both PSCs Ca^{2+} responses and synaptic plasticity of terminals. This indicates that PSCs detect synaptic transmission of competing terminals trough P2Y1Rs and, in turn, control synaptic plasticity. This plasticity is mediated by adenosine A_2A receptors as revealed by the use of the specific $A2_ARs$ antagonist SCH58261. Finally, chronic *in vivo* blockade of PSCs P2Y1 receptors resulted in an increased proportion of poly-innervated NMJs and delayed synaptic elimination at different post-natal ages. These results suggest that PSCs Ca^{2+} responses are required for synaptic plasticity of competing terminals and may influence the outcome of synaptic competition and connectivity.

Disclosures: **H. Darabid:** None. **R. Robitaille:** None.

Poster

666. Synapse Refinement

Location: Hall A

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

Support: University at Buffalo Rehabilitation Science Collaborative Grants to KEP

Title: NMDA receptor signaling at the neuromuscular junction accelerates developmental synapse elimination

Authors: *K. E. PERSONIUS[1], S. B. UDIN[2];
[1]Rehabil. Sci., [2]Physiol. and Biophysics, Univ. at Buffalo, Buffalo, NY

Abstract: During normal development, motor neurons form superfluous synaptic connections with muscle fibers. After birth, this multiple innervation is pared until each muscle fiber is innervated by a single motor neuron. The elimination of extraneous synapses is controlled by differential activity levels, with the less active input being eliminated. Acetylcholine has previously been thought to be the primary mediator of this activity, but here we show that N-methyl-D-aspartate (NMDA) receptors at the neuromuscular junction are pivotal modulators of synapse elimination. We have previously reported that synapse elimination is slowed by reducing glutamate receptor activation; thus we now tested whether the converse is true - that synapse elimination could be accelerated by increasing glutamate receptor activation. We implanted slices of a slow-release polymer (Elvax) infused with NMDA (0.1 mM) within the leg of P4 mice. The contralateral hindlimb was implanted with saline-infused Elvax to serve as an internal control. At P8, immunostaining revealed a significant increase in the rate of synapse elimination in the NMDA- vs saline-infused muscles. For the extensor digitorum longus (EDL) muscle the percentage of multiply innervated junctions was 37.5 ± 3.9 vs. 47.5 ± 3.4% for NMDA and saline treated legs, respectively ($n = 8$, $p < 0.05$; t-test). The soleus muscle showed a similar result (48.9 ± 4.2 vs. 63.5 ± 2.3% for NMDA and saline treated legs, respectively; $n = 9$, $p < 0.01$; t-test). We used calcium imaging to assess neuromuscular responses to NMDA (0.5 mM) in P4-to-adult mice. Bath application of NMDA resulted in increased neuromuscular calcium signaling in pups up to 3 weeks of age. Calcium signaling was blocked by APV (0.2 mM). Taken together, these results point to a role for NMDA receptor signaling during the activity-dependent phase of developmental synapse elimination at the neuromuscular junction.

Disclosures: **K.E. Personius:** None. **S.B. Udin:** None.

Poster

666. Synapse Refinement

Location: Hall A

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

Support: NIH 5R01NS031651

Title: Activity-dependent molecular mechanisms underlying synaptic refinement at the *Drosophila* NMJ

Authors: *F. J. VONHOFF[1], H. KESHISHIAN[2];
[1]Molecular, Cellular, and Developmental Biol., [2]MCDB Dept., Yale Univ., New Haven, CT

Abstract: Precise connectivity in neural networks relies on the strengthening of correct synaptic contacts and the removal of off-target synapses. Neural activity plays a crucial role in the refinement of neural circuits in vertebrates and invertebrates. For example, in the vertebrate visual system, low frequency calcium (Ca) and cyclic nucleotide oscillations are involved in the refinement of early projection maps. At the *Drosophila* neuromuscular junction (NMJ) synaptic refinement also occurs in an activity-dependent manner, where oscillatory neural activity and presynaptic Ca signaling modulate the motoneuron's response to the retrograde chemorepellent Sema2a for the removal of off-target contacts in a CaMKII-dependent fashion. We previously showed that mutations in the Ca-dependent adenylyl cyclase rutabaga as well as the cAMP-dependent phosphodiesterase dunce increase the frequency of miswired, ectopic neuromuscular synapses. Targeted RNAi knockdown and rescue experiments indicate a presynaptic role of these two genes, which regulate intracellular cAMP levels. Using the photoactivatable adenylyl cyclase bPAC, we show that presynaptic cAMP levels are required to oscillate within an optimal range. Activation of bPAC in an oscillatory pattern of 15 s light followed by a rest period of 150s darkness suppresses the miswiring phenotype observed in rut1 mutants. No rescue is observed using activation patterns of shorter cycles of 8s light: 80s darkness or longer cycles of 30s light:300s darkness. We tested the role PKA as a downstream target of cAMP. PKAIIR homozygous mutants show an increased frequency of ectopic contacts of 24% as compared to a frequency of 14% observed in controls. A similar miswiring phenotype is observed by the panneuronal expression of PKAIIR-RNAi. We are now testing the role of the protein-phosphatase1 (pp1) family in synaptic refinement, which serves as a molecular link between CaMKII and PKA in mammalian cells. We find that heterozygous double mutants for the pp1-87B and pp1a-96A genes show an increased ectopic frequency of 21.5%. Panneuronal RNAi knockdown of either gene phenocopies the observed effect. In order to monitor Ca activity during synaptic refinement, we used paralyzed embryos expressing the Ca reporter GCaMP5. Low frequency Ca oscillations are evident in native and ectopic contacts during synapse development. We are now using different FRET-based cAMP sensors for live imaging of cyclic nucleotide levels at motoneuron growth cones. Our results show that as in vertebrates, synaptic refinement in *Drosophila* is dependent on dynamic second messenger signaling pathways that engage both Ca and cyclic nucleotide oscillations

Disclosures: F.J. Vonhoff: None. **H. Keshishian:** None.

Poster

666. Synapse Refinement

Location: Hall A

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

Support: NIH 5R01NS031651

Title: Target-dependent retrograde signaling mediates synaptic plasticity at the *Drosophila* neuromuscular junction

Authors: *B. A. BERKE[1,2], H. KESHISHIAN[2];
[1]MCDB Dept., [2]Molecular, Cellular, and Developmental Biol. Dept., Yale Univ., New Haven, CT

Abstract: Neurons often innervate multiple postsynaptic cells with distinct synaptic strengths and expressions of activity-dependent plasticity. How can neural activity strengthen one synapse while leaving the innervation of other cells unaffected? Here we describe differential target-specific synaptic plasticity in *Drosophila* 'common exciter' (CE) motoneurons, which synapse onto multiple larval muscle fibers. By driving transgenes in only one of the CE-innervated muscle fibers, the other target muscles acted as controls to compare responses to elevated CE activity. Control NMJs expanded in the expected activity-dependent fashion (boutons increased in number by 15%, $p<0.005$). However, when the level of functional postsynaptic glutamate receptors (GluR, GluRC and GluRIIA) was reduced by RNAi or a dominant-negative transgene, the manipulated NMJ failed to expand ($p=0.3$), despite showing comparably robust spontaneous activity (8 Hz, $p=0.4$ with respect to control NMJs). This suggests that each muscle fiber can independently influence the growth and plasticity of its NMJ through a putative retrograde signal. A key retrograde transynaptic molecule necessary for growth and plasticity is the Bone Morphogenetic Protein (BMP) Glass bottom boat (Gbb). When Gbb was expressed in a single CE-innervated muscle fiber in a gbb mutant background, synaptic growth was rescued only for that NMJ (boutons reached 94% of WT, compared to 30% in non-rescued muscle fibers). Conversely, RNAi-mediated suppression of Gbb in that same muscle fiber blocked activity-dependent growth plasticity compared to other CE-innervated muscle fibers ($p<0.005$). This is intriguing, as Gbb promotes NMJ growth and plasticity through transcriptional regulation by the downstream SMAD1 transcription factor Mad. Activated Mad (pMad) is, however, found both in motoneuron nuclei and in presynaptic boutons at the NMJ. pMad immuolabeling was reduced at the CE-NMJ of the RNAi treated muscle fiber, yet no apparent change in pMad levels was observed in nuclei. Thus synaptic pMad may have a non-canonical function in tagging the NMJ to permit local growth and plasticity. Preliminary data also indicate that activity-dependent Ca signaling and Ca-dependent regulation of phosphorylation by CaMKII and Calcineurin play a role in this differential plasticity. We are currently examining the link between BMP signaling

and postsynaptic Ca and identifying additional molecules that are differentially expressed between manipulated and control presynaptic terminals.

Disclosures: **B.A. Berke:** None. **H. Keshishian:** None.

Poster

666. Synapse Refinement

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

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NIH/NINDS Grant 1R01NS076467-01

DOD Grant GG008784

NSF/NIH/JHU Grant 2001668272

Title: Quantifying synaptic reorganization in the developing cerebellum using serial section scanning electron microscopy data

Authors: ***A. M. WILSON**, R. SCHALEK, A. SUISSA-PELEG, T. R. JONES, S. KNOWLES-BARLEY, J. W. LICHTMAN;
Harvard Univ., Cambridge, MA

Abstract: Neurons undergo dramatic changes in connectivity during development. In both the peripheral and central nervous system some axons prune branches to innervate fewer postsynaptic target cells and each postsynaptic target cell is innervated by fewer axons. As synaptic connections are removed, surviving inputs are strengthened by additional synapses, resulting in a more focused connectivity with smaller numbers of neurons driving smaller numbers of target cells. This process, called synapse elimination, is thought to be regulated by synaptic activity and therefore experience. This phenomenon has been studied in parts of the peripheral nervous system using optical microscopy, but the small sizes and dense, tenuous arrangements of cells in the central nervous system has prevented identification of the neuronal branches or synapses of single inputs during development. In order to overcome this barrier to exploration of synaptic rewiring in the central nervous system, we have used serial section

scanning electron microscopy to produce 3D volumes of high-resolution images from cerebella of CD1 mice in early postnatal development. Unlike other methods, the resolution of electron microscopy is sufficient to clearly identify all synapses in a tissue sample. We use a recent adaptation of this technique in which a long series of thin sections is cut, collected on tape, and automatically imaged with scanning electron microscopy to generate images of thousands of thin sections with minimal loss and reasonably quickly. We have focused on the developing cerebellum because it undergoes a large scale change in connectivity and is intrinsically simpler than cerebral cortex. We have reconstructed neonatal Purkinje cells and their climbing fiber inputs at several developmental ages. We show that climbing fibers form many synapses onto somatic spines of immature Purkinje cells during the first week of life, and many synapses from parallel fibers and other cerebellar cell types are present at this same time. We also see that climbing fibers innervate immediately adjacent Purkinje cells during development, unlike the more distributed connections in adults. We see that synapses from single climbing fibers occupy territories on Purkinje cells that are largely segregated to different somatic regions by postnatal day 7.

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Poster

666. Synapse Refinement

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 666.10/B66

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

Support: Grant-in-Aid for Scientific Research (KAKENHI) No 25000015 from JSPS, Japan

Title: Neuroligin-2 regulates postnatal development of climbing fiber-Purkinje cell synapses in the cerebellum

Authors: ***E. LAI**, N. UESAKA, M. KANO;
The Univ. of Tokyo, Tokyo, Japan

Abstract: Formation of mature functional neural circuits depends on proper elimination of early-formed redundant synapses during postnatal development. It is also known that proper balance of excitation and inhibition is essential for establishing functional neural circuits. Neuroligin 2 (NL2) is an inhibitory synapse-specific cell adhesion molecule that functions in GABAergic synapse formation and stabilization through binding to presynaptic neurexins. Mice lacking NL2

have been reported to exhibit a selective perturbation of inhibitory synaptic transmission, but it remains unclear whether the NL2 deficient mice show any abnormality in developmental refinement of neural circuits. In the present work, we examined the effects of NL2 deletion on synapse elimination of redundant climbing fiber (CF) to Purkinje cell (PC) synapses in developing mouse cerebellum, a representative model of developmental refinement of neural circuits. We found that global NL2 knockout (KO) mice displayed both an increased expression of NL3 and a significantly decreased expression of GABAA receptor α1 subunit in the cerebellum. The amplitude of miniature inhibitory synaptic currents (mIPSCs) in PCs was reduced from P7 to adulthood, but excitatory synaptic transmission was normal. Because of this enhanced excitation/inhibition ratio, dendritic Ca2+ transients elicited by stimulating weaker CFs in individual PCs were significantly larger in NL2 KO than in wild-type mice, whereas dendritic Ca2+ transients by stimulating the strongest CFs were the same between the two genotypes. Importantly, elimination of redundant CF synapses was significantly impaired at P10 and thereafter in NL2 KO mice. In addition, PC-specific knockdown of NL2 by RNA interference significantly impaired CF synapse elimination, which was very similar to the phenotype of NL2 KO mice. These results strongly suggest that NL2 in PCs maintains functional balance between excitatory and inhibitory synapses and that this balance is crucial for properly eliminating redundant CF synapses from P10 and for establishing mature CF innervation of PCs.

Disclosures: **E. Lai:** None. **N. Uesaka:** None. **M. Kano:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.01/B67

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH NIDA Grant DA016176

Title: Cotinine, the major metabolite of nicotine, alters trafficking and assembly of nicotinic acetylcholine receptors

Authors: *A. M. FOX, F. H. MOONSCHI, C. I. RICHARDS;
Chem., Univ. of Kentucky, Lexington, KY

Abstract: Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels consisting of a combination of alpha (α2-10) and beta (β2-4) subunits. Regulated subcellular localization and stoichiometry are crucial for correct intracellular processing and function.

Exposure to nicotine alters the trafficking and assembly of nAChRs, resulting in an upregulation of number of receptors on the plasma membrane and a shift in the distribution of stoichiometry to favor the high sensitivity $(\alpha4)_2(\beta2)_3$ version. Although the mechanism of these processes is not fully understood, nicotine-induced upregulation is believed to contribute to nicotine addiction. We have found that cotinine, the primary metabolite of nicotine, also effects nAChR trafficking and assembly, meaning nicotine metabolites may have a previously unacknowledged role in the mechanism of nicotine addiction. We utilize a pH sensitive variant of GFP, super ecliptic pHluorin (SEP), to differentiate between intracellular nAChRs and those expressed on the plasma membrane to quantify changes resulting from cotinine and nicotine exposure. SEP is fluorescent at physiological extracellular pH 7.4, but off at a pH of less than 6. This allows us utilize pH differences in the cell to pinpoint receptor location, number, and resolve single vesicle insertion events at the membrane. Similar to nicotine, exposure to cotinine increases the number of α4β2 receptors on the plasma membrane and causes a redistribution of intracellular receptors. This can be partially attributed to an increase in number of insertion events when exposed to cotinine. In contrast to this, cotinine exposure down regulates α6β2β3. In addition to subcellular location, determining individual receptor stoichiometry is an important aspect in the development of diagnostics and therapeutics for smoking cessation. We used single molecule fluorescence studies to evaluate stoichiometry differences under nicotine or cotinine exposure. In these studies, a GFP that undergoes bleaching when exposed to continuous excitation is encoded in each alpha subunit sequence. Therefore, the number of single molecule bleaching steps detected corresponds to the number of alpha subunits in a nAChR pentamer, and thus the stoichiometry. We found that both nicotine and cotinine alter the assembly of α4β2 receptors to favor the high sensitivity $(\alpha4)_2(\beta2)_3$ stoichiometry. In conclusion, the nicotine metabolite, cotinine, also alters trafficking and assembly of nAChRs. Since cotinine's half-life is approximately ten times longer than nicotine, a potential role in nicotine addiction is feasible but not explored.

Disclosures: **A.M. Fox:** None. **F.H. Moonschi:** None. **C.I. Richards:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.02/B68

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant R21 DA026627 (P.W.)

Barrow Neurological Foundation Start-up Funds (P.W.)

Barrow Neurological Foundation Fellowship (M.M.W.)

Title: Nocturnal frontal lobe epilepsy-associated intracellular-loop mutant subunits alter single-channel properties of alpha4beta2-nicotinic receptor isoforms

Authors: *M. M. WELTZIN, A. A. GEORGE, R. J. LUKAS, P. WHITEAKER;
The Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Nocturnal frontal lobe epilepsy (NFLE) is a group of familial seizure disorders associated with mutations in nicotinic acetylcholine receptor (nAChR) subunits. alpha4beta2-nAChRs are the most prevalent central nervous system subtype. They exist as two isoforms with distinctive subunit stoichiometries and high or low sensitivity to nicotinic agonists [HS (alpha4)2(beta2)3- and LS (alpha4)3(beta2)2-nAChR, respectively]. They are abundantly expressed in the thalamocortical network, an area highly implicated in epilepsy. Two novel NFLE-associated mutations have been identified in the alpha4 and beta2 subunit cytoplasmic loop 2 (C2) domains. The C2 domain is an under-studied region of nAChR subunits, and mutations in this region have previously not been linked to NFLE. We expressed either alpha4beta2-nAChR isoform by injecting Xenopus oocytes at alpha4:beta2 unlinked subunit cRNA ratios of 1:10 or 30:1 (HS or LS receptors, respectively) or as concatenated (linked subunit), pentameric receptors (HSP or LSP). Concatenated subunits ensure pure expression of a single isoform, enabling comparison to results collected using biased ratio injections of unlinked subunit cRNAs. There is close resemblance of receptor isoform properties whether expressed from linked or unlinked subunits. Single-channel patch-clamp recordings were used to define unitary responses to acetylcholine (ACh) at specific concentrations. Parameters examined include unitary amplitude, channel conductance, open and closed dwell times, probability of the channel being open, event lifetimes and events per burst. We found significant parameter differences between alpha4beta2-isoforms and effects of alpha4 or beta2 C2 domain mutant subunit incorporation. We determined that wildtype loose subunit and concatenated LS-isoform receptors have two unitary amplitudes (LSP: 0.98 ± 0.03 and 1.76 ± 0.06 pA), while wildtype HS and HSP receptors have one unitary amplitude (HSP: 1.51 ± 0.04 pA). NFLE C2 domain mutations minimally altered the unitary amplitudes with the exception of the beta2 mutant subunit reducing the HS-isoform amplitude (1.13 ± 0.09 pA). Expression of the beta2 mutant subunit in the LS-isoform significantly increased the open-dwell time of the longest component (wildtype: $\tau 2 = 1.48 \pm 0.13$ vs. beta2 mutant: $\tau 2 = 2.40 \pm 0.10$ ms). Other single channel parameters were also affected by the mutant subunits. These studies indicate the functional consequences of NFLE-associated mutations at the single-channel level that illuminate bases for C2 domain-mediated alterations in receptor function and may help explain how those changes relate to disease pathology.

Disclosures: **M.M. Weltzin:** None. **A.A. George:** None. **R.J. Lukas:** None. **P. Whiteaker:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.03/B69

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant GM 48677

NIH Grant GM103801

Title: Characterization of nicotinic acetylcholine receptors formed by gain of function α6 subunit

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Abstract: α6* nAChRs (* denotes presence of other subunits) have gained recent attention due to their role in dopamine release that is a hallmark of substance use reinforcement and addiction. α6* nAChRs are also preferentially lost in striatal regions of animal models of Parkinson's and in humans with Parkinson's disease. In the dopaminergic system, the α6 subunit combines with the β2 and β3 subunits and in some instances the α4 subunit to form a functional receptor. Unique chaperones may allow in-vivo assembly of α6β2β3* nAChRs. Obtaining adequate expression levels of functional α6β2* nAChRs in heterologous systems such as Xenopus oocytes and transfected cell lines has proven extremely difficult. Progress has been made by using subunit chimeras, point mutants of the α6 or β3 subunit or concatameric subunits. Gain-of-function mutations in the α6 and/ or β3 subunit have also been employed with some success. In this study, we are investigating mutations of residues in the TM2 region of the human and rat α6 subunits. The human α6L9'A subunit forms functional nAChRs in Xenopus oocytes when expressed with the β2 and β3 subunits. A panel of α-conotoxins showed a pharmacology consistent with that obtained when using chimeric α6/α3 subunit. The use of minimally altered but functional α6 subunits may prove useful in efficient expression of heterologously expressed α6* nAChRs and subsequent development of α6β2* drugs.

Disclosures: **L. Azam:** None. **J.M. McIntosh:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH F30 DA036312

NIH F31 DA029386

NIH R21 DA033543

NIH R21 DA031952

Title: Modulation of nicotine reward-associated behaviors by mir-542-3p

Authors: *A. CASSERLY, L. LIU, R. ZHAO-SHEA, E. HOGAN, M. SCOFIELD, A. R. TAPPER, P. D. GARDNER;
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Abstract: Nicotine binds to and activates a family of ligand-gated ion channels, neuronal nicotinic acetylcholine receptors (nAChRs). Chronic nicotine exposure alters the expression of various nAChR subtypes, which likely contributes to nicotine dependence; however, the underlying mechanisms regulating these changes remain unclear. A growing body of evidence indicates that microRNAs (miRNAs) may be involved in nAChR regulation. Using bioinformatics, miRNA library screening, site-directed mutagenesis and gene expression analysis, we have identified a limited number of miRNAs that functionally interact with the 3′-untranslated regions (3′-UTRs) of mammalian nAChR subunit genes. miRNAs typically regulate gene expression via direct interactions with specific, evolutionarily conserved sites (miRNA recognition elements [MREs]) within the 3′-UTRs of their targets. In silico analysis identified a number of MREs within the 3′-UTRs of the nAChR subunit genes. Mutating these sites disrupted miRNA regulation thereby confirming the in silico predictions. Through this screen we identified the β2 nAChR subunit as a target of miR-542-3p and confirmed that miR-542-3p down-regulates its expression *in vitro* and *in vivo*. We show that miR-542-3p is expressed in the mouse ventral tegmental area (VTA), a component of the mesocorticolimbic reward circuitry. In addition, miR-542-3p expression in the VTA is regulated by chronic nicotine exposure. Finally, overexpression of miR-542-3p in the VTA blocks a conditioned place preference to nicotine. Our results provide evidence for a novel mode of regulation of β2 nAChR subunit expression by

nicotine. Furthermore, our data suggest a key role for miRNAs in the modulation of nicotine-reward associated behaviors.

Disclosures: **A. Casserly:** None. **L. Liu:** None. **R. Zhao-Shea:** None. **E. Hogan:** None. **M. Scofield:** None. **A.R. Tapper:** None. **P.D. Gardner:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

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Topic: B.02. Ligand-Gated Ion Channels

Support: Arizona Biomedical Research Commission

Barrow Neurological Foundation

National Institutes of Health

Title: Differential modulation of α3β4 and α3β4α5 nAChR isoforms by the endogenous neuromodulator lynx1

Authors: ***A. A. GEORGE**, B. EATON, R. J. LUKAS, P. WHITEAKER;
Neurobio., The Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Nicotinic acetylcholine receptors (nAChRs) exist as pentameric complexes of homologous, but genetically-distinct subunits. Recent studies have demonstrated that α3β4* nAChRs (*indicating the presence of additional subunits) mediate mechanisms in the medial habenula (MHb) circuitry that regulate negative reward processing. The α5 nAChR subunit can integrate into these complexes, and the α5 subunit D398N variant has been implicated in increased susceptibility to nicotine dependence, perhaps by lowering nicotine aversion. Endogenous neuropeptides, such as lynx1, can influence subunit stoichiometry, cell-surface expression and function of α4β2-containing nAChRs. However, the functional consequences of lynx1 interaction with α3β4* or α3β4α5* nAChRs are poorly understood. In the current study, we used a concatenated (i.e. linked) subunit approach to ensure consistent subunit incorporation while probing the functional modulation of α3β4* and α3β4α5* nAChRs by lynx1. Using two-electrode voltage clamp (TEVC) electrophysiology, we demonstrate that lynx1 attenuates macroscopic receptor peak currents for (α3β4)2α3, (α3β4)2β4, (α3β4)2α5[D398] and (α3β4)2α5[N398] subtypes when expressed in Xenopus oocytes. Radio-immmunolabeling reveals that lynx1 also significantly reduces cell surface levels of (α3β4)2β4, (α3β4)2α5[D398]

and (α3β4)2α5[N398] nAChR cell-surface expression but not of (α3β4)2α3 nAChRs. Single-channel recordings confirm preferential functional effects of lynx1 on (α3β4)2α3-containing nAChRs, reducing single-channel conductance and burst percentage. Significant temporal shifts in single-channel closed dwell-times were also observed for (α3β4)2α3 nAChRs, indicating the ability of lynx1 to enhance (α3β4)2α3 single-channel desensitization. No statistically-significant changes in single-channel parameters were observed for any other α3β4* or α3β4α5* nAChR isoform. Given the established role of α3β4* and α3β4α5* nAChR function in the habenulo-peduncular pathway, it seems likely that selective modulation of α3β4* nAChRs by lynx1 could represent a valuable strategy in modulating neuronal activity in brain areas involved in reward processing. This work was supported the Arizona Biomedical Research Commission's Early Investigator Award (A.A.G) and by NIH grants R21 DA027070 and R21 DA036059S (to P.W).

Disclosures: **A.A. George:** None. **B. Eaton:** None. **R.J. Lukas:** None. **P. Whiteaker:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant GM085237

Title: Functional impact of 13 single nucleotide polymorphisms causing missense mutations of human α7 nicotinic receptor

Authors: *Y. CHANG[1], Q. ZHANG[2], Y. DU[3], Y. HUANG[4], R. J. LUKAS[1];
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Abstract: The α7 nicotinic receptor (nAChR) is a major subtype of the nAChRs in the central nervous system, and the receptor plays an important role in brain function. In the dbSNP database, there are 55 single nucleotide polymorphisms (SNPs) that cause missense mutations of the human α7nAChR in the coding region. In this study, we tested the impact of 13 SNPs that cause missense mutations in the agonist binding site or the coupling region between binding site and channel gate on the receptor function. The wild type or mutant receptors were expressed or co-expressed in *Xenopus* oocytes, and the agonist-induced currents were tested using two-electrode voltage clamp. Our results demonstrated that 6 mutants were nonfunctional, 5 mutants

had reduced current expression, and some of them had slightly reduced agonist sensitivity. Interestingly, the function of 4 out of 6 nonfunctional mutants could be rescued by α7nAChR positive allosteric modulator PNU-120596. Finally, when coexpressed with the wild type, the nonfunctional mutants could also influence the receptor function. These changes of the receptor properties by the mutations could potentially have impact on physiological function of the α7nAChR-mediated cholinergic synaptic transmission and anti-inflammatory effects in the human SNP carriers. Rescuing the nonfunctional mutants could provide a novel way to treat the related disorders.

Disclosures: **Y. Chang:** None. **Q. Zhang:** None. **Y. Du:** None. **Y. Huang:** None. **R.J. Lukas:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.07/B73

Topic: B.02. Ligand-Gated Ion Channels

Title: The prototoxin LYPD6B modulates heteromeric alpha3 beta4 containing nicotinic acetylcholine receptors (nAChRs) but not alpha7 homomers

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Abstract: Prototoxins are a diverse family of membrane-tethered molecules expressed in the nervous system. Several prototoxins modulate nicotinic signaling, but the functional significance of this is not clear. We identified a new prototoxin, LYPD6B, and tested its selectivity and efficacy on α3(α5)β4 versus α7-containing nicotinic acetylcholine receptors (nAChRs). To constrain stoichiometry, fusion proteins encoding human α3, β4, and α5 (D and N variants) heteromers were expressed in Xenopus oocytes with LYPD6B. Controls lacked LYPD6B. We used two-electrode voltage-clamp to quantify responses to ACh: sensitivity (EC50), maximum current induced (Imax), and rate of desensitization (τ). LYPD6B acts selectively on (α3)3(β4)2 and (α3)2(α5D)(β4)2 leaving unaffected (α3)2(β4)3, α7 and (α3)2(α5N)(β4)2 nAChRs. For β4α3α3β4α3 and β4α3β4α3α3, LYPD6B decreases EC50 from >631 μM to approximately 100 μM, reduces Imax significantly by at least 59%, and decreases τ. For β4α3α5Dβ4α3 and β4α3β4α3α5D, LYPD6B decreases Imax by 63% and 32% respectively. For β4α3α5Dβ4α3 and β4α3β4α3α5D, LYPD6B decreases Imax by 63% and 32% respectively. Thus, LYPD6B is a

positive modulator of (α3)3(β4)2 at low [ACh] but a negative modulator at high [ACh]. LYPD6B also negatively modulates α3β4 nAChRs that include the α5 D variant, but not the N variant associated with smoking dependence.

Disclosures: V. Ochoa: None. **P. Whiteaker:** None. **A.A. George:** None. **R. Nishi:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

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Program#/Poster#: 667.08/B74

Topic: B.02. Ligand-Gated Ion Channels

Title: Chimeras of first transmembrane domain identify important residues for expressing extracellular domain α4β2 nicotinic acetylcholine receptors

Authors: *G. B. WELLS, A. M. GALVAN, A. M. PERSON;
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Abstract: Background: Extracellular domain receptors formed from truncated Cys-loop subunits are potentially valuable for structural and functional studies. Their smaller size and reduced transmembrane content focuses investigations on properties of ligand-binding extracellular domain and might lead to higher resolution X-ray crystallographic structures than can be achieved with full length receptors. Uncertainty persists about how the first transmembrane domain (M1) and specific residues in M1 contribute to expressing extracellular domain Cys-loop receptors. What is the best sequence for M1 for expressing a given extracellular domain Cys-loop receptor? **Objective**: To help answer this question, the goal was to identify important residues in M1 through chimeric constructs of M1 from α7 nicotinic acetylcholine receptor (nAChR) and serotonin 5-HT3A receptor (5-HT3AR) subunits. These chimeras were designed in the background of extracellular domain α4M1/β2M1 nAChRs. **Methods**: Human α4 and β2 cDNAs were truncated after M1 (α4M1 and β2M1). M1 sequences from chicken α7 nAChR and mouse 5-HT3AR subunits were swapped into α4M1 and β2M1. Roles of specific residues in M1 were probed by changing residues from the α7 nAChR subunit into residues from the 5-HT3AR subunit based on side chain properties and conservation that was evident through multiple sequence comparisons. Subunits were expressed as cRNA in *Xenopus laevis* oocytes. Immunoblotting and yield of [^{3}H]epibatidine binding sites assessed effects of the changes. **Results**: The α7 chimera of α4M1/β2M1 according to [^{3}H]epibatidine binding showed high structural fidelity to extracellular domain α4M1/β2M1 and full length α4β2 nAChRs. The α7 chimera had significantly higher expression than the 5-HT3AR chimera.

Swapping at positions in M1 with different hydrophobic side chains, which were expected to be freely interchangeable, instead decreased expression of [^{3}H]epibatidine binding sites. Swapping at positions of M1 with side chains of different polarity, ionization properties, chemical reactivity, or bulk decreased expression of [^{3}H]epibatidine binding sites. Swapping residues along nearly the entire M1 affected expression of [^{3}H]epibatidine binding sites. **Conclusions:** M1 functions as more than a nonspecific, transmembrane tether for the extracellular domains of α4M1/β2M1 nAChRs. These results suggest that specific residues of M1 might have specific structural and functional roles for extracellular domain Cys-loop receptors. These results help refine the concept of an optimally designed M1 sequence for expressing a given extracellular domain Cys-loop receptor.

Disclosures: G.B. Wells: A. Employment/Salary (full or part-time):; Texas A&M University. **A.M. Galvan:** A. Employment/Salary (full or part-time):; Texas A&M University. **A.M. Person:** A. Employment/Salary (full or part-time):; Texas A&M University.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: B.02. Ligand-Gated Ion Channels

Support: BFU2012-30997

MH-53631

GM-48677

Title: Differential effects of varenicline and nicotine on endogenous α3β4* nicotinic acetylcholine receptors in human adrenal chromaffin cells

Authors: *A. ALBILLOS[1], L. RUEDA-RUZAFA[1], J. M. MCINTOSH[2], J. PASSAS[3], C. DE CASTRO-GUERIN[4], J. BLAZQUEZ[5], C. GONZALEZ-ENGUITA[6], A. J. HONE[1]; [1]DEPARTAMENTO DE FARMACOLOGÍA (L6), UNIVERSIDAD AUTONOMA DE MADRID, MADRID, Spain; [2]UNIVERSITY OF UTAH, SALT LAKE CITY, UT; [3]HOSPITAL DOCE DE OCTUBRE, MADRID, Spain; [4]HOSPITAL LA PAZ, MADRID, Spain; [5]HOSPITAL CLINICO SAN CARLOS, MADRID, Spain; [6]FUNDACION JIMENEZ DIAZ, MADRID, Spain

Abstract: Nicotine and varenicline are known to activate heterologously expressed human α3β4 nicotinic acetylcholine receptors (nAChRs) but very little information is available concerning their activities on endogenously expressed receptors in native human cells. We used patch-clamp electrophysiology to assess the activities of nicotine and varenicline on α3β4 nAChRs expressed in human adrenal chromaffin cells. Under voltage-clamp conditions both nicotine and varenicline evoked whole-cell currents in these cells but varenicline was more potent than either nicotine or acetylcholine (ACh). In current-clamp mode, continuous perfusion of EC_{50} concentrations of nicotine (20 μM) and varenicline (4 μM) initially increased the number of ACh-evoked action potentials (APs) and were capable of evoking APs in the absence of ACh stimulation. Perfusion of varenicline (50-100 nM) at concentrations achieved in humans robustly increased the number of ACh-evoked APs whereas nicotine (50-250 nM) did not. Higher concentrations of varenicline (250-500 nM) further increased the number of ACh-evoked APs whereas nicotine (500 nM) inhibited them. Our results suggest that nicotine and varenicline may both activate and desensitize human adrenal chromaffin cell α3β4 nAChRs which may alter the release of catecholamines from adrenal chromaffin cells. These results provide valuable information concerning the pharmacology of endogenously expressed human adrenal chromaffin cell α3β4 nAChRs and may help guide the development of therapeutic compounds used in the treatment of nicotine addiction.

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Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

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Topic: B.02. Ligand-Gated Ion Channels

Support: IPRS scholarship

Title: Exploiting ligand selectivity to understand allosteric receptor opening upon agonist binding

Authors: *D. INDURTHI, T. BALLE, P. AHRING, M. CHEBIB, N. ABSALOM;
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Abstract: Introduction: Nicotinic acetylcholine receptors (nAChR) are cation ion channels and are implicated in several mental illnesses. α4β2 nAChR exists in two stoichiometries, (α4)2(β2)3 and (α4)3(β2)2, with distinct pharmacological properties, due to the presence of additional agonist binding site at α4-α4 interface on (α4)3(β2)2 receptor. Although very little is known, determining molecular mechanism of receptor opening is important for understanding receptor functioning. Methodology: Using two-electrode voltage clamp technique we measured responses of sazatidine-A and TC-2559 on both wild-type and mutant α4β2 nAChRs, to test ligand selectivity for different binding interface. Kinetic modelling was used to determine mechanism of receptor opening.<br Result and discussion: Sazatidine-A and TC-2559 acted as partial agonists on (α4)3(β2)2 but as full agonists on (α4)2(β2)3 wild-type receptors. When tested on (α43m)3(β2)2 mutant receptor that is engineered to only have the α4-β2 agonist binding site, both compounds act as full agonists, suggesting their interaction at α4-β2 interface on α4β2. However, no activity was observed on (α4)3(β23m)2 mutant receptor that is engineered to have only α4-α4 binding interface, therefore demonstrating ligand selectivity for α4-β2 and not at α4-α4 binding interface. When these compounds were co-applied with NS9283 that binds only at the α4-α4 binding interface, full activation was observed on wild-type (α4)3(β2)2 receptors. This activation profiles were used to propose a kinetic model that best represents an activation model for heteromeric nAChRs.

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Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: B.02. Ligand-Gated Ion Channels

Title: Critical determinants of alpha7 nAChR allosteric activation and modulation: pharmacological agents and structural epitopes that separate those activities

Authors: *G. A. THAKUR[1], R. PAPKE[2], A. KULKARNI[1], N. HORENSTEIN[3]; [1]Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA; [2]Dept. of Pharmacol. and Therapeut., [3]Dept. of Chem., Univ. of Florida, Gainesville, FL

Abstract: Critical determinants of α7 nAChR allosteric activation and modulation: pharmacological agents and structural epitopes that separate those activities. The α7 nicotinic acetylcholine receptor is of current interest as a drug target for indications ranging from

cognitive disorders to inflammation. Under normal conditions, α7 nicotinic acetylcholine receptor (nAChR) ion channels activate less readily than do those of other nAChR subtypes. However, the unique properties of α7 receptors render them sensitive to selective positive allosteric modulators (PAMs), which increase the efficiency of channel activation to a level greater than that of other nAChR. By definition, PAMs must work in concert with "orthosteric" agonists such as ACh, while compounds such as GAT107, the active isomer of 4BP-TQS, have the combined properties of agonists and PAMs, able to function as single agents to produce very effective channel activation (ago-PAMs). Ago-PAMs activate α7 nAChR ion channels far more effectively than ACh, or other orthosteric agonists working at the same binding site as ACh, in the absence of a PAM. The direct activation of receptors by ago-PAMs arises from the effects of these agents at two different sites. One site, likely to be within the receptor's transmembrane domains, is likely to be the same as where PAMs like PNU-120596 function to promote the enhanced activation by orthosteric agonists, destabilizing nonconducting states associated with orthosteric desensitization by reducing the barrier for transition into a novel conductive state. The other site, likely to be solvent accessible in the extracellular domains, is a unique site for allosteric activation (AA) of PAM-potentiated receptors. One phenomenological distinction we observe for the two sites is that more rapid washout and exchange with solution tends to be observed for the AA site. Low GAT107 concentrations, are relatively ineffective at the PAM site, however, activity at the AA site can be increased by PNU-120596 binding to the PAM site. We identify key attributes of GAT107 required for activity at the AA site through the characterization of two different classes of competitive antagonists of AA that do not inhibit PAM activity. We have also identified several mutations in α7 which disable activation by orthosteric agonists These mutants remain sensitive to allosteric activation by GAT107 and that activity is also blocked by the same antagonists which block the allosteric activation of wild-type α7. Analysis of key molecular features of effective allosteric agonists and antagonists allows us to propose a model for recognition features within the AA site and identify putative binding sites.

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Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant 5 R25 NS080687

Title: Modulation of the alpha 7 nicotinic acetylcholine receptor by ibuprofen

Authors: *J. C. RODRIGUEZ, J. O. COLON-SAEZ, J. A. LASALDE DOMINICCI;
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Abstract: Members of the nicotinic acetylcholine receptor family (nAChR) like the α7 nAChR have been linked to the inflammatory process making it a possible target for anti-inflammatory treatments. Recent studies have shown that inflammation is related to the development of various diseases like cancer, diabetes, asthma and Alzheimer's disease. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) commonly used to treat inflammation, fever and pain. The objective of these experiments was to determine whether ibuprofen can modulate the activity of the α7 nAChR. This was achieved by using Xenopus oocytes injected with mRNA coding for the α7 nAChR. The oocytes were incubated for a 48 hours period to allow for channel expression. The functionality and the amplitude of the response of the α7 nAChR was determined using the two electrode voltage clamp technique on Xenopus oocytes. The amplitude of the responses was determined in the presence and absence of ibuprofen. In the presence of ibuprofen, the amplitude of the α7 nAChR's response was significantly reduced. This suggests a possible role for ibuprofen in the modulation of the α7 nAChR function. Further studies will be directed at determining ibuprofen's mechanism of action and whether other members of the nAChRs family are also modulated by it. Also, we will determine if other common drugs used to treat inflammation and pain, like acetaminophen and naproxen, are also able to modulate the function of neuronal nAChRs.

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Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

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Support: NIH Grant DA012976

Georgetown University

Title: Nicotinic acetylcholine receptor subtypes expressed in the rodent habenula

Authors: *R. VENKATESH, R. P. YASUDA, T. H. GUPTA-GOLDENBERG, B. B. WOLFE, K. J. KELLAR;
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Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) play a critical role in nicotine addiction. In mammalian nervous systems, heteromeric nAChRs are composed of combinations of 8 alpha and 3 beta subunits. These combinations constitute receptor subtypes, each of which has distinct biophysical and pharmacological properties. nAChRs are highly expressed in the habenula, and are of particular interest because the subtypes expressed there may be associated with sensitivity/tolerance to nicotine in animals allowed to freely self-administer the drug (Fowler et al., Nature, 2011,) and with symptoms of withdrawal in animals treated chronically with nicotine (Salas et al., J Neurosci, 2009.) Although the rat habenula expresses several nAChR subunits (Grady et al., J Neurosci, 2009; Scholze, J Neurochem, 2012,) the receptor subtypes they form are not entirely clear. In this study, we used immunoprecipitation and radioligand binding methods to investigate the nAChR subtypes expressed in the rat habenula. In particular, we used sequential immunoprecipitation assays to determine the extent to which two subunits are associated. Our data indicate that both β2 and β4 subunits are highly expressed in the habenula (at about 50% and 60%, respectively) and that they are associated with each other in about 20% of receptors. Additionally, both the β2 and β4 subunits are associated with α3, α4, and α5 subunits. Our study also indicated a significant population of α3α5β2β4* receptors; however, in contrast to several other brain regions, we found no associations between the α4 and α5 subunits in the rat habenula. [3H]-Epibatidine saturation binding in habenular tissue fit a single site with a Kd of 78 pM, but competition binding assays with nicotine in habenular tissue fit a two-site model. The nAChR subtypes in the habenula are likely to be important. In agreement with Grady et al. (2009), our studies show that the habenula is rich in “uncommon” nAChR subtypes; consistent with this, immunoprecipitation and competition binding studies indicate a diverse population of receptors. These data may help identify the habenula nAChRs involved in nicotine addiction and withdrawal, which may aid in the development of more effective smoking cessation drugs.

Disclosures: R. Venkatesh: None. **R.P. Yasuda:** None. **T.H. Gupta-Goldenberg:** None. **B.B. Wolfe:** None. **K.J. Kellar:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.14/B80

Topic: B.02. Ligand-Gated Ion Channels

Title: The role of calcium and calcium-sensitive signaling pathways in nicotine-induced upregulation of alpha 7 receptors expressed in xenopus oocytes

Authors: *K. DEBOEUF[1], M. ISLAM[2], J. PANCHAL[3], J. ROSE[4], J. FARLEY[2]; [1]Psychology, [2]Neurosci., [3]Biol., Indiana Univ., Bloomington, IN; [4]Ctr. for Smoking Cessation, Duke Univ. Med. Ctr., Durham, NC

Abstract: The α7 neuronal nicotinic-acetylcholine receptors (α7 nAChRs) play important roles in learning, memory, nicotine-addiction/withdrawal, and many other neurological conditions. Nicotine-produced upregulation of α7 Rs is believed to be involved innicotine-addiction and relapse, but reliable upregulation of α7 and its underlying mechanism(s) are poorly characterized. In our recent studies of α7 Rs heterologously expressed in Xenopus oocytes (see accompanying abstract), we have described the procedural determinants that lead to reliable, 2-fold increases of α7 currents by 12-14 hrs of exposure to 100 μM nicotine followed by extensive washout. Because of the large Ca2+ conductance of α7 Rs, intra- and/or extracellular Ca2+ and Ca2+ -sensitive signaling pathways might play a critical role in such upregulation. In our studies, nicotine-upregulation of α7 Rs was unaffected by elimination of extracellular Ca2+ (zero Ca2+ plus EGTA). However, intracellular Ca2+ chelation (by BAPTA-AM) completely blocked nicotine-upregulation, without affecting basal α7 current expression, or de novo expression of Kv1.1 channels. Several Ca2+ -dependent signaling pathways were critical for nicotine-upregulation: PP2B/calcineurin (inhibited by cyclosporin A), Ser/Thr protein kinase-activity (inhibited by H7), and possibly PKC isozymes (activated by PMA). 12-14 hr incubation with 10 μM CsA + 100 μM nicotine, yielded a mean current of 756.46 ± 137.66 nA (n=15), which was significantly greater ($p < 0.05$) than control currents of 307.65 ± 59.40 nA (n=15), but clearly sub-additive for the CsA alone controls (512.04 ± 142.0 nA, n=20) and nicotine alone oocytes (501.43 ± 99.04 nA, n=23). Similarly, a combination of H7 +nicotine produced significant ($p < .05$) upregulation (511.09 ± 91.14 nA, n=16), that did not exceed that produced by 25 μM H7 alone (560.79 ± 121.17 nA, n=15), nor that produced by nicotine alone. Thus, both CsA and H7 largely occluded nicotine-upregulation of α7.12-14 hr exposure to 100 nM PMA + 100 μM nicotine strongly suppressed α7 currents [24.94 ± 5.54 nA (n=5)], vs same-batch controls and nicotine alone oocytes (679.28 ± 99.05 nA, n=10), suggesting that PKC-inhibition by nicotine might be involved in upregulation. However, PMA's strong suppression of α7 currents precluded a clear interpretation of PKC's involvement. In contrast, although protein tyrosine kinase (PTK) activity (inhibited by genistein) had large effects on basal α7 currents (via both PAM- and non-PAM effects), PTKs did not seem to participate in nicotine-upregulation. Our results suggest that Ca2+-dependent inhibition of PP2B and one or more Ser/Thr kinases underlies nicotine-upregulation.

Disclosures: K. Deboeuf: None. **M. Islam:** None. **J. Panchal:** None. **J. Rose:** None. **J. Farley:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.15/B81

Topic: B.02. Ligand-Gated Ion Channels

Support: Faculty Development Fund of TAMHSC

Title: 3-(2-chlorophenyl)-5-(5-methyl-1-(piperidin-4-yl)-1H-pyrrazol-4-yl)isoxazole is a selective positive allosteric modulator of low-sensitivity (α4)3(β2)2 nicotinic acetylcholine receptor

Authors: *A. K. HAMOUDA[1,2], Z.-J. WANG[1], T. S. MOHAMED[1], A. B. ALASKARI[1]; [1]Pharmaceut. Sci., Texas A&M Hlth. Sci. Ctr., Kingsville, TX; [2]Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Positive allosteric modulators (PAM) of nicotinic acetylcholine receptors (nAChR) are a class of drugs that allow selective modulation of a subpopulation of brain nAChRs. However, structural information necessary for the design of nAChR subtype-selective PAMs is lacking and studies are needed to identify binding sites for nAChR PAMs and to define the molecular determinants of PAMs nAChR-subtype selectivity. To this end, we use site-directed mutagenesis coupled with *in-vitro* electrophysiological recording to identify binding site(s) for a novel α4β2 nAChR PAM, 3-(2-chlorophenyl)-5-(5-methyl-1-(piperidin-4-yl)-1H-pyrrazol-4-yl)isoxazole (CMPI); Albrecht et al. 2008, Biorg. Med. Chem. Lett. 18: 5209). Recording from *Xenopus* oocytes injected with low-sensitivity $(\alpha4)_3(\beta2)_2$ or high-sensitivity $(\alpha4)_2(\beta2)_3$ nAChRs, we found that CMPI selectively potentiates ACh-induced current of $(\alpha4)_3(\beta2)_2$ but not $(\alpha4)_2(\beta2)_3$ nAChR. For $(\alpha4)_3(\beta2)_2$ nAChR, CMPI produced a left-shift of ACh dose-response curve without altering ACh efficacy. At 10 μM ACh, CMPI potentiated the response of $(\alpha4)_3(\beta2)_2$ maximally by ~400% with an EC50 of ~1 μM. For $(\alpha4)_2(\beta2)_3$ nAChRs, CMPI produced a dose-dependent inhibition of ACh response with a maximal inhibition of 30% at 10 μM CPMI. Mutational analyses of amino acids contributing to the α4:α4 interface, which is present in the $(\alpha4)_3(\beta2)_2$ but not the $(\alpha4)_2(\beta2)_3$ nAChR, identified amino acids necessary for CMPI potentiation that are distinct from those identified as determinants of potentiation by NS9283, another $(\alpha4)_3(\beta2)_2$ nAChR selective PAM (Olsen et al 2013, *JBC 288*: 35977). CPMI potentiation was not affected by α4H114V substitution (α4H142V when numbering with signal peptide included) that reduced potentiation by NS9283. On the other hand, substitutions at α4K62 and α4E64 abolished potentiation by CMPI without affecting NS9283 potentiation. Further mutational analyses are ongoing to refine the definition of the location of PAM binding site(s) at the α4:α4 interface.

Disclosures: **A.K. Hamouda:** None. **Z. Wang:** None. **T.S. Mohamed:** None. **A.B. Alaskari:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.16/B82

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS077114

Title: Expression and purification of the intracellular domain of an anionic pentameric ligand-gated ion channel

Authors: A. PANDHARE, *M. JANSEN;
Cell Physiol. Mol. Biophys., TTUHSC, Lubbock, TX

Abstract: Anionic pentameric ligand-gated ion channels (pLGIC) of the Cys-loop superfamily are receptors for neurotransmitters such as gamma-aminobutyric acid (GABA) and glycine. These receptors are the targets for a number of currently used clinical drugs including general anesthetics, anti-epileptics (anti-convulsants), sedatives, anxiolytics, and muscle relaxants. Towards developing new improved drugs as well as improving current treatments, it is imperative to determine how each of these pLGICs functions, more specifically, to identify and characterize the different structural elements that mediate each aspect of receptor function. All Cys-loop receptor subunits in metazoans contain three domains: an extracellular domain (ECD), a transmembrane domain (TMD), and an intracellular domain (ICD). The functional roles of ECD and TMD have been extensively studied and their three-dimensional structures have been determined. The identification of prokaryotic pLGICs, e.g. *Gloeobacter violaceus* ligand-gated ion channel (GLIC), lead to structural information at atomic resolution. However, the prokaryotic members lack the ICD. Interestingly, the ICD of Cys-loop receptors from the animal kingdom is the most diverse domain with respect to both length and amino-acid composition. The ICD, therefore, represents an attractive target for developing subtype-selective drugs with the promise of fewer side effects than current drugs, which all target the highly-conserved extracellular or transmembrane domains. Therefore, the main goal of our project was to develop a large-scale protein expression and purification strategy to produce the ICD of an anionic pLGIC. To this end, we have generated chimeras by inserting the ICD of the $GABA_A\rho1$ receptor into proteins of known X-ray structures. We have optimized conditions for expression in *E. coli* culture, as well as purification to homogeneity, yielding 10-15 mg of purified protein per L

culture. Thus, our results are consistent with the successful large-scale expression and purification of the $GABA_A\rho$1-ICD chimera which is now amenable to structural studies.

Disclosures: A. Pandhare: None. **M. Jansen:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.17/B83

Topic: B.02. Ligand-Gated Ion Channels

Support: Spanish Ministerio de Ciencia y Tecnología [BFU2012-30997 to A.A.]

U.S. National Institutes of Health [MH-53631 and GM-48677 to J.M.M]

A.J.H holds a Marie Curie International Fellowship from the European Commision.

Title: Species difference pharmacology of native α3β4* nicotinic acetylcholine receptors in rat, cow, and human adrenal chromaffin cells

Authors: *L. RUEDA[1], A. J.HONE[2], J. MCINTOSH[3], J. PASSAS[4], A. ALBILLOS[2];
[1]Univ. Autónoma De Madrid, Madrid, Spain; [2]Univ. Autónoma de Madrid, Madrid, Spain; [3]Univ. of Utah, Salt Lake, UT; [4]Hosp. 12 de Octubre, Madrid, Spain

Abstract: Adrenal chromaffin cells from rat and cow have previously been reported to express α3β4 nicotinic acetylcholine receptors (nAChRs) yet debate surrounds the functional expression of β2-containing nAChRs in these cells. Here we present pharmacological evidence demonstrating that adrenal chromaffin cells from these two species express few functional nAChR that contain β2 ligand-binding sites and that the predominant subtype expressed is α3β4. Patch-clamp electrophysiology was used to evaluate the activites of several subtype-selective α-conotoxins (α-Ctxs) on the ACh-evoked currents. The α3β4 and α6β4 selective α-Ctx TxID completely blocked ACh-evoked currents in both rat and cow cells indicating that the nAChRs mediating these currents contain the β4 subunit. Furthermore, the ACh-evoked currents were insensitive to α-Ctxs selective for either α6- or β2-containing nAChRs suggesting that there were few receptors present with these subunits. Together, these results demonstrate that the predominant nAChR expressed in these cells is the α3β4 subtype. Subsequent experiments were performed using rat, cow, and human cells to evaluate the potencies and efficacies of several nAChR agonists. Potencies for ACh, nicotine, varenicline, cytisine, and dimethyl-piperazinium were all similar among the three species however agonist efficacies were strikingly different.

Cytisine was a low efficacy agonist in bovine cells but a full agonist in rat and human. Varenicline behaved as a full agonist in human cells but a partial agonist in rat and cow cells. Finally, nicotine was a full agonist in cow cells but a partial agonist in human and rat cells. These studies highlight the importance of species difference considerations when evaluating the activities of both agonists and antagonists of α3β4 nAChRs.

Disclosures: L. Rueda: None. **A. J.Hone:** None. **J. McIntosh:** None. **J. Passas:** None. **A. Albillos:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.18/B84

Topic: B.02. Ligand-Gated Ion Channels

Support: Oxford Brookes University PhD scholarship

Title: The β2/β2 interface of the (α4β2)2β2 nicotinic acetylcholine receptor does not bind competitive ligands

Authors: K. NEW, *I. BERMUDEZ, S. MAZZAFERRO;
Oxford Brookes Univ., Oxford OX3 0BP, United Kingdom

Abstract: Nicotinic acetylcholine receptors (nAChRs) containing α4 and β2 subunits (α4β2 nAChRs) are the most abundant heteromeric nAChRs in the brain. These are of particular interest due to converging evidence indicating that α4β2 nAChRs are a key mediator of the reinforcing effects of nicotine. α4β2 nAChRs have also been implicated in a wide range of brain functions, including cognition, mood and nociception. α4 and β2 nAChR subunits assemble into alternate stoichiometries (α4β2)2α4 and (α4β2)2β2 that display remarkable differing sensitivity to activation by agonists and allosteric modulators. Recently, it has been shown that an additional operational agonist site is present in the α4/α4 interface of the (α4β2)2α4 nAChR, and this site accounts for the ligand-selectivity and desensitisation pattern of this receptor stoichiometry. To probe whether a competitive ligand binding site is present in the β2/β2 subunit interface of the (α4β2)2β2 nAChR, we have alanine substituted conserved aromatic residues that contribute to competitive ligand binding in canonical agonist binding sites in nAChRs. To avoid uncertainties in data interpretation due to potential assembly and expression of multiple receptor forms, we carried out these studies on fully linked (α4β2)2β2 nAChRs. We find that mutations expected to impair competitive ligand binding in canonical agonist sites have no impact on receptor function.

We conclude from these findings that competitive ligands do not bind the β2/β2 interface, in contrast to what has been demonstrated for the α4/α4 interface in the (α4β2)2α4 nAChR.

Disclosures: K. New: None. **I. Bermudez:** None. **S. Mazzaferro:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.19/B85

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant T32HD007491

Title: ACR-16 nAChRs are actively transported to synapses by a CDC-42 dependent pathway

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

Abstract: The delivery of nicotinic acetylcholine receptors (nAChRs) to synapses is essential for the proper formation, maintenance, and plasticity of cholinergic neural circuits. Using an *in vivo* approach in the model organism *C. elegans*, our lab has previous shown that α7-type nAChRs are dependent on a non-canonical Wnt signaling pathway for translocation to the cell surface from subsynaptic stores. This pathway is specific to ACR-16/ α7 nAChRs in that mutations in Wnt signaling components result in decreased nicotine-gated current and subsynaptic accumulations of ACR-16::GFP but do not affect neighboring levamisole-gated AChRs or GABA receptors. Little is known however, about how receptors are transported to these subsynaptic stores following synthesis. We now have evidence that actin polymerization is necessary for the trafficking of ACR-16/ α7 nAChRs to muscle arms. Using real-time *in vivo* imaging and fluorescence recovery after photobleaching (FRAP) experiments, we can dissociate between receptors that move to synapses through passive diffusion versus those that are transported actively in an ATP-dependent manner. We find that this process is dependent on a myosin 1D motor and is regulated by the Rho GTPase CDC-42. This work outlines a pathway for efficient delivery of nAChRs to synapses that may be regulated by activity and disrupted in diseases that affect cholinergic signaling.

Disclosures: A.J. Kallarackal: None. **J. Mellem:** None. **D. Madsen:** None. **A. Maricq:** None.

Poster

667. Nicotinic Acetylcholine Receptors: Structure and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 667.20/B86

Topic: B.02. Ligand-Gated Ion Channels

Title: Nicotine induced upregulation of alpha 7 nicotinic acetylcholine receptor expressed in xenopus oocytes: key factors and determinants of upregulation

Authors: *M. ISLAM[1], K. DEBOEUF[1], P. B. SCHWARTZ[2], T. MURUGESAN[3], J. ROSE[3], J. FARLEY[1];
[1]Neurosci., [2]Sch. of Med., Indiana Univ. Bloomington, Bloomington, IN; [3]Ctr. for Smoking Cessation, Duke Univ. Med. Ctr., Durham, NC

Abstract: Alpha 7 (α7 Rs), a major subtype of nAChRs appear to play critical roles in learning, memory, and various neuropathologies including nicotine addiction. Nicotine-upregulation of α7 Rs is thought to play significant roles in these phenomena. But whether nicotine-upregulation of α7 Rs reliably occurs, and the nature of its underlying mechanism(s), are largely unknown. Previous *in vitro* studies of α7 nAChRs heterologously expressed in Xenopus oocytes failed to observe nicotine-upregulation. These failures might have been due to incomplete removal of nicotine from the recording media, as a result of its intracellular accumulation and subsequent slow release from the oocytes, resulting in desensitization of α7 Rs during functional assays. In our experiments, 12-14 hr exposure to nicotine (100 μM) 4-5 days post cRNA-injection (PI), followed by extensive washout yielded statistically-significant ~2-fold increases in α7 currents [614.25 ± 53.44 (mean ± SEM) nA, n=95; $t(200) = 5.97$, $p < 0.001$] as compared to controls (294.36 ± 19.6 nA, n= 107), as determined by TEVC and α7-protein (by Western blot). Net charge was also ~ 2X bigger in nicotine-treated cells vs. controls, but the kinetic indices (net charge/peak) were similar. Less-extensive washout, as well as 100 nM nicotine incubation, failed to produce upregulation. Instead, inactivated/desensitized currents were observed. Direct GC/MS measurement of nicotine in the washout fluid confirmed that nicotine was continually released from oocytes and the concentration was ~20 nM at 3-4 hr following onset of washout, well in excess of the ~ 3nM IC50 value. Exposure of α7 oocytes to cumulative washout fluid produced suppressed α7 currents (23.59 ± 10.35 nA, n=9) as compared to controls (295.58 ± 25.53 nA, n=14). Nicotine-upregulation did not depend on new protein synthesis, as cycloheximide (1 μM) affected neither the maintained expression of 4-5th day α7 currents nor nicotine- upregulation. On the 5th day PI, the magnitude of the nicotine-upregulated currents was correlated with the level of basal expression of α7 Rs, but the extent of upregulation was remarkably consistent: ~2X. However, early in the course of expression (2-3 days PI cRNA), 100 μM nicotine failed to produce upregulation. Similar to nicotine, methyllycaconitine, a cell-permeable competitive

antagonist of α7 Rs, and carbachol, a stable membrane-impermeable agonist, also produced ~ 2X-upregulation. However, ACh incubation (which can be hydrolyzed by the oocytes) failed to produce upregulation. These results suggest that a persistent ligand-binding to α7 Rs (but not necessarily gating of the ion channels) was critical for α7 Rs upregulation.

Disclosures: M. Islam: None. **K. DeBoeuf:** None. **P.B. Schwartz:** None. **T. Murugesan:** None. **J. Rose:** None. **J. Farley:** None.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.01/B87

Topic: B.02. Ligand-Gated Ion Channels

Support: VCU Massey cancer Center

Title: Antiallodynic effects of substituted N-aryl piperidinium salts: alpha7 nAChR silent agonists

Authors: N. HORENSTEIN[1], D. BAGDAS[3], M. QUADRI[1], *M. DAMAJ[3], R. L. PAPKE[2]; [1]Chem., [2]Pharmacol. and Therapeut., Univ. of Florida, Gainesville, FL; [3]Dept. of Pharmacol. & Toxicology, Virginia Commonwealth Univ., Richmond, VA

Abstract: The α7 nicotinic acetylcholine receptor (nAChR) is an emerging target for treatment of conditions associated with inflammatory processes. In a number of cases, ligands that are active in this way do not mediate observable ion currents, but are desensitizers of ionotropic activity. We term compounds of this type “silent agonists”, and have previously shown that the silent agonist NS6740 has α7-dependent analgetic activity in several pain models (Papke et al., 2015). The MQ series of substituted analogs of N,N-diethyl N’-phenylpiperazinium (diEPP) have been generated (see presentation by Horenstein et al. at this meeting). In this study we evaluated the antiallodynic activity of several MQ compounds that were silent agonists of α7 nAChR in the CFA mouse model, a model of chronic inflammatory pain. When applied alone, none of these compounds activated either heteromeric nAChR or α7 expressed in Xenopus oocytes. To varying degrees they were able to activate α7-mediated currents when co-applied with the positive allosteric modulator (PAM) PNU-120596, identifying them as silent agonists. In regards to their efficacy for stimulating PAM-dependent currents we found the following trend: 2.MQ.95 > 2.MQ.79 > 2.MQ.75. The compound 2.MQ.75 is a tertiary amine while the other two are quaternary amines, likely to have limited blood brain permeability. After systemic

administration (i.p.) in male ICR mice treated with CFA, 2.MQ.95 and 2.MQ.75 were active in reversing CFA-induced allodynia in a dose-related manner. However, 2.MQ.79 failed to show any antiallodynic effect in the mouse. These results suggest that a hard charge on the piperidine nitrogen is not a critical feature for antiallodynic activity.

Disclosures: N. Horenstein: None. **D. Bagdas:** None. **M. Quadri:** None. **M. Damaj:** None. **R.L. Papke:** None.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.02/B88

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH GM57481

Title: Synthesis and structure activity relationships for α7 nAChR silent agonism in N-phenylpiperazinium salts

Authors: *N. HORENSTEIN[1], M. QUADRI[2], C. STOKES[3], R. PAPKE[3];
[1]Chem., Univ. Florida, Gainesville, FL; [2]Chem., [3]Pharmacol. and Therapeut., Univ. of Florida, Gainesville, FL

Abstract: The α7 nicotinic acetylcholine receptor (nAChR) is a ligand gated ion channel widely distributed in the nervous system and peripheral tissues, even in leukocytes. It is a potential target in a number of processes ranging from neurocognition to inflammation. Conventional pharmacological thought places emphasis on control of this receptor via agonists, partial agonists and antagonists. Interestingly, α7 desensitization can be converted to a conductive state with a positive allosteric modulator (PAM) such as PNU-120596, and we have identified compounds that are selective in their ability to place the receptor in a PAM sensitive desensitized state (Ds) without significant channel activation. We define these compounds silent agonists (Papke et al., 2014), and they are associated with anti-inflammatory effects (see posters by Gould et al, Damaj, et al). The MQ series of compounds are substituted analogs of diethylphenylpiperazinium (diEPP) which itself is an analog of the ganglionic agonist dimethylphenylpiperazinium (diMPP). While diMPP is an efficacious orthosteric agonist of ganglionic (α3β4) heteromeric nAChR and homomeric α7 nAChR, when applied alone, diEPP will not activate these receptors, but places them into Ds. We synthesized a number of compounds in the MQ series with the key reaction being Pd-catalyzed coupling of piperazines with aryl compounds. The substituent on the

phenyl ring was varied in both identity and position and we assayed the compounds on the α7 and α3β4 nAChRs as expressed in the Xenopus oocyte using TEVC methodology. Across the series we observed variations in the ability of compounds to activate α7 as partial agonists with most exhibiting barely detectable activity while many produced robust responses when co-applied with PNU-120596 indicating they were selective for desensitization. The emergent four most probative compounds featured electron dense grouping at the para position of the phenyl ring including fluoro, trifluoromethyl and carboxamide groups. We hypothesize that polar interactions at the para position are key to silent agonism by favoring conversion of the bound complex into a PAM-sensitive desensitized state. A subpopulation of the receptors will also to varying degree reside in a PAM insensitive desensitized state (Di). The relative induction of these states may be probative for functional aspects of these compounds. Additionally, some compounds in the series exhibited noncompetitive inhibition of α3β4 nAChRs. The data presented here are part of a larger picture in which we seek to define the design features required for silent agonists, define state distributions, and apply them for control of the α7 nAChR *in vivo*.

Disclosures: **N. Horenstein:** None. **M. Quadri:** None. **C. Stokes:** None. **R. Papke:** None.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.03/B89

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant GM57481

Title: Insights into an emerging class of α7 nAChR silent agonists and NF-κB signaling mechanisms in immune cells

Authors: *T. M. GOULD[1], M. QUADRI[1], N. A. HORENSTEIN[1], R. L. PAPKE[2];
[1]Chem., [2]Pharmacol. & Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Compounds that preferentially stabilize non-conducting, desensitized α7 nicotinic acetylcholine receptor (nAChR) conformations (e.g. silent agonists) are potential therapeutic candidates for inflammatory and pain-related disorders. We have identified a new class of α7 nAChR silent agonists (the MQ series) with *in vitro* anti-inflammatory activity. The MQ compounds are derived from diethylphenylpiperazinium (diEPP), the parent α7 nAChR silent agonist. The lead MQ compounds inhibited stimulus-induced NF-κB activation in Jurkat and THP-1 cells, most with an approximate IC50 of 50 μM and some with no significant effects on

cell viability at concentrations up to 1 mM. Collaborative studies on the MQ series are also described in abstracts by Horenstein et al. and Damaj et al., which characterize the synthesis, electrophysiological properties, and *in vivo* efficacy of these agents. We also studied the mechanism(s) by which drugs targeting α7 nAChR impact the regulatory state of NF-κB in THP-1 cells. We tested whether drugs that differentially impact LPS-stimulated NF-κB activity (GTS-21 and NS6740) can affect the phosphorylation and degradation of IκBα, a protein that binds to cytosolic NF-κB thereby inhibiting its transcriptional activity. GTS-21 and NS6740 also differentially altered the phosphorylation state and level of IκBα, consistent with the reciprocal effects the drugs had on NF-κB activity. We conclude that these drugs modulate NF-κB activity by altering the phosphorylation state and (presumably) degradation of IκBα at the NF-κB/IκBα complex. We hypothesize that α7 drug-mediated regulation of NF-κB is catalyzed by drug-induced perturbations to receptor conformation, which could facilitate transient protein-protein interactions at intracellular domains, and may involve the recruitment of signaling proteins with SH2 domains (e.g. Src kinases, PI3K, JAK2). We speculate that such interactions either alter transmission of the canonical LPS/TLR4/NF-κB signal transduction pathway, or activate other pathways that can impact the regulatory state of IκBα. Together these studies provide insights into a novel class of α7 nAChR silent agonists with anti-inflammatory activity and the molecular mechanism(s) of nAChR drug-mediated NF-κB signaling.

Disclosures: **T.M. Gould:** None. **M. Quadri:** None. **N.A. Horenstein:** None. **R.L. Papke:** None.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.04/B90

Topic: B.02. Ligand-Gated Ion Channels

Support: ISU Seed Grant

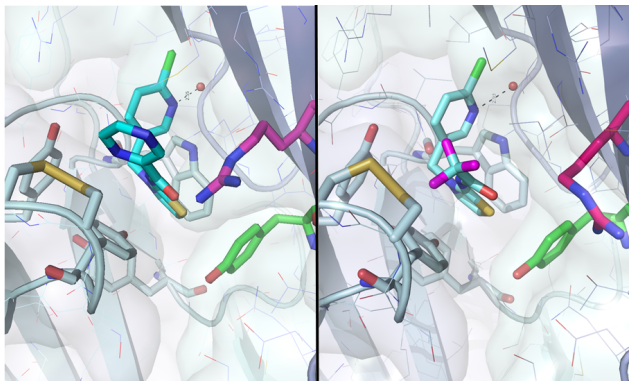
Aslam Foundation

COP SU Funds

Title: Chimeric acetylcholine binding protein for structure-guided insecticide design

Authors: *J. BOBANGO, T. T. TALLEY;
Dept. of Biomed. and Pharmaceut. Sci., Idaho State Univ., Meridian, ID

Abstract: The biological target of the fastest growing class of insecticides worldwide are the nicotinic acetylcholine receptors (nAChRs). Structural characterization of the binding interactions of insecticides with target and off-target species has not yet been examined. This is potentially a concern due to selectivity and resistance issues. Further, insect nAChRs to date have been notoriously difficult to heterologously express. Current expression and assay techniques use truly hybrid receptors or a heterogeneous mix of the binding target from crude tissue preparations. Here we present the soluble acetylcholine binding protein (AChBP) from the mollusk *Aplysia* californica as an alternative means for exploring insecticidal structural characterization. This soluble protein has been established as a structural surrogate for the native receptor and has the potential to be utilized for high-throughput screening. Additionally, this protein is amenable to the development of chimeric binding proteins relative to species of interest. These chimeras are generated by introducing mutations in key loops in the ligand binding domain. In the current study, we have produced chimeric AChBPs to mimic specific insect species nAChRs with mutations introduced in the integral loop C region and in the loop D region. The insect species we have completed mutations for include: the model organism *Drosophila* melanogaster (fruit fly), Myzus persicae (green peach aphid), Heliothis virescens (moth), Anopheles gambiae (mosquito), and the off-target honey bee Apis mellifera. In addition, we have completed chimeric constructs relative to humans. This will allow the comparison of binding interactions elucidated by x-ray crystallography between target and off-target species and provide data to drive structure-guided drug design. This combined with radioligand competition binding assays will aid the development of novel, selective, and safer insecticides.



Disclosures: **J. Bobango:** None. **T.T. Talley:** None.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.05/B91

Topic: B.02. Ligand-Gated Ion Channels

Support: WSU COP Startup Funds

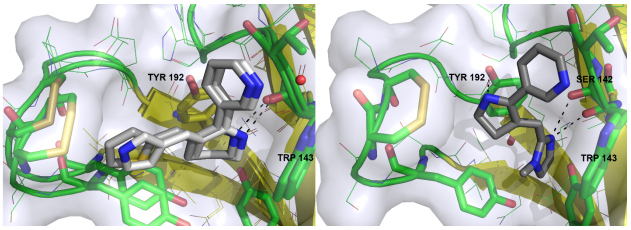
ISU COP Startup Funds

ALSAM Foundation

Title: Structure-activity guided design and analysis of arylidene anabaseines and myosmines reveal two distinct binding modes with the acetylcholine binding protein

Authors: *T. T. DENTON[1], T. T. TALLEY[2], J. BOBANGO[2];
[1]Washington State Univ. Col. of Pharm., Spokane, WA; [2]Biomed. and Pharmaceut. Sci., Idaho State Univ. Col. of Pharm., Meridian, ID

Abstract: Nicotinic acetylcholine receptors (nAChRs) are recognized for playing a pivotal role in cognitive function. For more than a decade now, the acetylcholine binding proteins (AChBPs), soluble surrogates of the membrane bound nAChRs, have been extensively used as screening tools and for the X-ray crystallographic determination of ligand-binding interactions between natural products and synthetic compounds with the AChBPs. In an effort to identify new potential therapeutics, that may ultimately serve as leads in the development of new compounds to aid in the treatment of cognitive disorders, a number of arylidene anabaseines and myosmines were synthesized and screened using competition assays with the AChBPs. The most potent compounds were selected for co-crystallization and X-ray structural analysis. Most of the compound structures examined demonstrated protein ligand interactions similar to those seen in the structures of AChBPs with 2,4-dimethoxybenzylidene anabaseine (DMXBA). In these structures, as well as most examined to date, the basic nitrogen of the imine forms a hydrogen bond with the carbonyl of Trp143 (Ls numbering) deep within the binding pocket. Further, the distal ring of most of the compounds was oriented out of the binding packet often demonstrating pi - thio interactions with the vicinal cysteines at the tip of loop C. However, there was one significant outlier. The X-ray structure of (1-methyl-1H-imidazol-2-yl)methylidene myosmine uncovered a novel binding pose where the distal imidazole ring is twisted deep within the binding site. The nitrogen of the imidazole ring forms a hydrogen bond with both Trp143 and Ser142. In addition, a new hydrogen bond between the protonated imine nitrogen of the compounds central ring and the hydroxyl group of Tyr192 is observed. This novel binding pose has led to the development of a second generation of these compounds now in development.



Disclosures: **T.T. Denton:** None. **T.T. Talley:** None. **J. Bobango:** None.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.06/B92

Topic: B.02. Ligand-Gated Ion Channels

Support: ISU COP Startup Funds

WSU COP Startup Funds

ALSAM Foundation

Title: A survey of high resolution acetylcholine binding protein X-ray structures reveals details of tertiary and quaternary movements of the protein, both real and imagined, upon ligand binding

Authors: *T. T. TALLEY[1], T. T. DENTON[2], J. BOBANGO[1];
[1]Dept. of Biomed. and Pharmaceut. Sci., Idaho State Univ. Col. of Pharm., Meridian, ID; [2]Col. of Pharm, Washington State Univ., Spokane,, WA

Abstract: The acetylcholine binding proteins (AChBPs) have provided a wealth of information about the molecular organization of nicotinic receptors since the initial report by Sixma and colleagues in 2001. Currently there are more than 90 structures of the AChBPs deposited in the PDB. This impressive compilation of endeavors by many groups globally demonstrates the importance of the AChBPs in both increasing our understanding of protein/ligand interactions and in the development of novel compounds. During the past year our group has deposited a number of high resolution (better than 2.5 Å) structures of the AChBPs from both *Lymnaea stagnalis* (*Ls*) and *Aplysia californica* (*Ac*). In addition we have deposited a number chimeric entities in which specific segments of the proteins have been modified to resemble the corresponding regions of specific human receptor subtypes. Included in this set are examples of natural products, peptide toxins, and novel synthetic entities as well as the highest resolution

structure to date of the WT AChBP from *Ls*. A comparison of the higher resolution structures for our group and others demonstrate significant changes in both the tertiary and quaternary structure of the AChBPs dependent, to some degree, on the ligand bound. At the same time the increasing number of high quality structures bring into question some assertions made about the AChBPs in the past. In particular it is apparent that comparisons made between different species with different ligands must be considered with some skepticism due to the inherent differences in structure and specificity. Further, differences in the expression systems used and the expression/purification tags present result in subtle differences in quaternary organization of the AChBPs from the same species. These subtleties may have led to the over interpretation of structural differences in the past. Full consideration of both the benefits and limitations of the information presented by structures of the AChBPs provides a less biased interpretation of the data. A detailed comparison of these issues will be presented in an effort to provide the community with the tools needed to make an informed assessment of the AChBP structures available.

Disclosures: **T.T. Talley:** None. **T.T. Denton:** None. **J. Bobango:** None.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.07/B93

Topic: B.02. Ligand-Gated Ion Channels

Support: CTI Project nr. 13875.1 PFLS-LS

Title: Walking in the chemical space identifies a novel cognitive enhancer acting at the α7 nAChRs

Authors: ***D. C. BERTRAND**[1], J.-L. REYMOND[2], S. BERTRAND[1], T. SCHAER[1], F. MARGER[1], J. BÜRGI[2], P. M. CALLAHAN[3], A. TERRY[3];
[1]Hiqscreen, Vesenaz - GE, Switzerland; [2]Dept. of Chem. and Biochem., Berne, Switzerland; [3]Dept. of Pharmacol. & Toxicology, Augusta, GA

Abstract: The computer guided exploration of the yet unknown chemical space such as the Chemical Universe Database GDB-17 containing all possible small organic molecules up to 17 atoms,1 is one of the richest tools available today to identify new active molecules for a given target.2 Extending our previous drug discovery efforts at the nicotinic acetylcholine receptors,3 we have identified promising regions of the chemical space and refined our virtual screening

strategies, in particular focusing on optimizing pharmacological characteristics such as crossing the blood brain barrier. Functional testing using expression of the human α7 neuronal nicotinic acetylcholine receptors and counter screened at heteromeric nAChRs including the α4β2, α3β2, α3β4 or α1β1δε. Active molecules were further tested for their possible interaction with this serotoninergic receptors. Different molecules were retained through this screening strategy with the most promising HiQ0013 acting almost as full agonist the human α7 with an EC50 in the μM range. Further physiological characterization revealed that sustained exposure to HiQ0013 in the nanomolar range potentiates the acetylcholine-evoked currents through a mechanism referred as priming that was observed only for some α7 agonists. Priming of the α7 receptors is known to correlate with procognitive activity, which suggested that HiQ0013 might be a promising candidate to restore cognitive deficits. Experiments conducted in rodents (mice and rats) confirmed that exposure to low concentrations of HiQ0013 can restore pharmacologically induced cognitive deficits. Furthermore, HiQ0013 at 0.03 mg/Kg was shown to improve delay to match sample task in old monkeys. These data confirmed the procognitive nature of HiQ0013 opening promising avenues for novel therapeutic treatments. 1.Ruddigkeit, L.; van Deursen, R.; Blum, L. C.; Reymond, J. L., Enumeration of 166 billion organic small molecules in the chemical universe database GDB-17. J. Chem. Inf. Model. 2012, 52, (11), 2864-2875. 2.Reymond, J. L., The chemical space project. Acc. Chem. Res. 2015, 48, (3), 722-730. 3.Burgi, J. J.; Awale, M.; Boss, S. D.; Schaer, T.; Marger, F.; Viveros-Paredes, J. M.; Bertrand, S.; Gertsch, J.; Bertrand, D.; Reymond, J. L., Discovery of Potent Positive Allosteric Modulators of the alpha3beta2 Nicotinic Acetylcholine Receptor by a Chemical Space Walk in ChEMBL. ACS Chem. Neurosci. 2014, 5, 346-359.

Disclosures: **D.C. Bertrand:** A. Employment/Salary (full or part-time):; HiQScreen Sàrl. **J. Reymond:** None. **S. Bertrand:** A. Employment/Salary (full or part-time):; HiQScreen Sàrl. **T. Schaer:** A. Employment/Salary (full or part-time):; HiQScreen Sàrl. **F. Marger:** A. Employment/Salary (full or part-time):; HiQScreen Sàrl. **J. Bürgi:** None. **P.M. Callahan:** None. **A. Terry:** None.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.08/B94

Topic: B.02. Ligand-Gated Ion Channels

Title: Novel alpha 7 nicotinic acetylcholine receptor (nAChR) modulator, HiQ0013 exhibits pro-cognitive effects in young and aged animal models

Authors: *A. V. TERRY, JR[1], P. CALLAHAN[1], C. HERNANDEZ[1], M. PLAGENHOEF[1], J.-L. REYMOND[2], S. BERTRAND[3], J. BURGI[2], D. BERTRAND[3];
[1]Dept Pharmacol Toxicol, Georgia Regents Univ., Augusta, GA; [2]Chem. and Biochem., Univ. of Berne, Berne, Switzerland; [3]HiQScreen Sàrl, Geneva, Switzerland

Abstract: The alpha 7 nicotinic acetylcholine receptor (nAChR) has long been considered a therapeutic target for the cognitive deficits of disorders like Alzheimer's disease (AD) and schizophrenia. This premise is based on a number of observations including deficits in alpha 7-nAChRs in the post mortem brains of patients who suffered from AD or schizophrenia, the abundance of alpha 7-nAChRs in the hippocampus and prefrontal cortex (important structures for cognition) and the important role of alpha 7-nAChRs in modulating several calcium-dependent events in neurons including neurotransmitter release, postsynaptic signaling, and neuronal survival. In the experiments described here, the novel alpha 7-nAChR modulator, HiQ0013 was evaluated in several learning and memory-related behavioral tasks in animal models. These tasks included a spontaneous novel object recognition (NOR) procedure in young-adult (4-5 month old) rats (N=6), a delayed match to sample (DMTS) task in old monkeys (mean age = 22.8 years, N=8), and a contextual and cued fear conditioning procedure in old mice (mean age =27 months, N=8). In the NOR task, vehicle and three doses of HiQ0013 ranging from 0.3-3.0 mg/kg were administered i.p. 30 min before the A/A session and the A/B (recall) session was conducted 48 hr later. All 3 doses of HiQ0013 improved performance of the NOR task. In the DMTS task in old monkeys, five doses of HiQ0013 ranging from 0.01-1.0 mg/kg were administered i.m. 30 min before behavioral testing. In these studies three of the five doses administered were associated with improvement in matching accuracy most notably at the longest (i.e., presumably the most difficult) delays. In the contextual and cued fear conditioning task in old mice, one dose (3.0 mg/kg administered i.p 30 min before testing) has been evaluated to date and it was associated with improvements in both contextual and cued fear conditioning. Collectively, these studies in animals indicate that HiQ0013 has the potential to improve several domains of cognition that are often impaired in neuropsychiatric disorders such as AD and schizophrenia including recognition memory, working/short memory, and associative learning.

Disclosures: A.V. Terry: None. **P. Callahan:** None. **C. Hernandez:** None. **M. Plagenhoef:** None. **J. Reymond:** None. **S. Bertrand:** None. **J. Burgi:** None. **D. Bertrand:** None.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.09/B95

Topic: B.02. Ligand-Gated Ion Channels

Support: The Augustinus Foundation

Agnes and Poul Friis Foundation

Fonden til lægemiddelvidenskabens fremme

Title: Beta-amyloid and Lypd6 compete for binding to nicotinic acetylcholine receptors in human brain extracts

Authors: *M. ARVANITI[1], J. D. MIKKELSEN[2], M. S. THOMSEN[1];
[1]Univ. of Copenhagen, Copenhagen, Denmark; [2]Neurobio. Res. Unit, Copenhagen Univ. Hosp. Rigshospitalet, Copenhagen, Denmark

Abstract: Nicotinic acetylcholine receptors (nAChRs) are widely expressed in the nervous system, affecting major brain processes, including learning and memory, and are implicated in neurodegenerative disorders, such Alzheimer's disease (AD). The alpha7 (α7) nAChR has been established as a promising molecular target for the treatment of AD, and clinical trials have shown that nAChR agonists are able to mitigate symptoms of the disease, improving attention and memory. Moreover, beta-amyloid (Aβ1-42), the main component of amyloid plaques in brains of AD patients, binds to α7 nAChR with high affinity. Ly-6/neurotoxin (Lynx) proteins, a group of neuronal modulators structurally similar to neurotoxins, regulate nAChR function in brain by binding directly to the receptors, critically affecting nAChR-dependent cognitive function. Recently our group demonstrated that PSCA, a member of the Lynx family, is altered in brains of AD patients and in an animal model of AD, implicating for the first time Lynx proteins in AD pathology. Here we investigate the Lynx protein Ly6/Plaur domain containing 6 (Lypd6), which modulates calcium conductance of nAChRs and enhances nicotinic-related behaviours, such as pre-pulse inhibition and hypoalgesia. Interestingly, Lypd6 is also shown to act as a feedback enhancer of Wnt/β-catenin signaling by binding directly to the essential co-receptor LRP6. Since Lypd6 modulates both cholinergic and Wnt/β-catenin signaling, two systems which are critically impaired in AD, we hypothesize that Lypd6 is involved in AD pathophysiology. In this study, we use affinity purification of human temporal cortex to show that Lypd6 binds to several nAChR subunits in human brain extracts. Using the same method, we demonstrate that oligomeric Aβ1-42 and Lypd6 compete for binding to nAChRs. Moreover, competition studies with nAChR ligands are performed to determine the binding site of Lypd6 on nAChRs. Lastly, we demonstrate that soluble recombinant Lypd6 protein reduces nicotine-induced ERK phosphorylation in PC12 cells. In summary, our data illustrate for the first time that Lypd6 binds to nAChRs in the human brain and that binding of Lypd6 is sufficient to inhibit nAChR-mediated signalling. Our findings further suggest a possible role of Lypd6 in AD. This finding may be used as a basis for more rational drug development for cognitive dysfunction.

Disclosures: **M. Arvaniti:** None. **J.D. Mikkelsen:** F. Consulting Fees (e.g., advisory boards); Advisory board member and part time consultant for Bionomics Ltd. **M.S. Thomsen:** F. Consulting Fees (e.g., advisory boards); Part time consultant for Bionomics Ltd.

Poster

668. Nicotinic Acetylcholine Receptors: Structure and Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 668.10/B96

Topic: B.02. Ligand-Gated Ion Channels

Support: Danish Research Council (COGNITO)

The Lundbeck Foundation

The Augustinus Foundation

Agnes and Poul Friis Foundation

Fonden til lægemiddelvidenskabens fremme

Title: Unravelling the alpha7 nicotinic acetylcholine receptor complex using affinity purification

Authors: ***M. S. THOMSEN**[1,2], E. N. LYUKMANOVA[3,4], M. A. SHULEPKO[3,4], J. D. MIKKELSEN[2];
[1]Dept. of Drug Design and Pharmacology, Univer, Copenhagen, Denmark; [2]Neurobio. Res. Unit, Copenhagen Univ. Hosp., Copenhagen, Denmark; [3]Shemyakin-Ovchinnikov Inst. of Bioorganic Chem., Russian Acad. of Sci., Moscow, Russian Federation; [4]Lomonosov Moscow State Univ., Moscow, Russian Federation

Abstract: Psychiatric and neurodegenerative diseases, such as Alzheimer's disease and schizophrenia, are characterized by disabling impairments of cognitive function, including memory lapses and lack of attention. The α7 (alpha 7) nicotinic acetylcholine receptor (nAChR) is one of the most promising molecular drug targets for cognitive disabilities. Previous research has largely considered the α7 nAChR to be a homomer in the brain. However, several lines of evidence suggest that α7 nAChR subunits interact directly other membrane proteins including other nAChR subunits. Here we provide evidence that the α7 nAChR forms complexes with several other proteins in human neocortical extracts: 1)We use affinity purification with α-bungarotoxin to demonstrate that α7 subunits can form complexes with β2 (beta2) nAChR subunits. Previous studies have shown that α7 and β2 subunits can form a heteromeric nAChR in

the rodent brain, and that this receptor is particularly vulnerable to blockage by Aβ, suggesting that effects on α7β2 nAChRs might underlie some of the cognitive dysfunction observed in AD. It has further been shown that existing α7 ligands vary in their efficacy on α7β2 receptors. Therefore the existence of the α7β2 nAChR may explain the differential clinical effects observed with "selective" α7 nAChR agonists. 2)We use affinity purification with α-bungarotoxin to demonstrate that α7 nAChRs can dimerize with the NMDA-receptor subunit NR1. This extends previous rat studies showing that α7 nAChRs can dimerize with NMDA-Rs by direct interaction with the NR2A subunit of the NMDA-receptor, and that disrupting this complex can alter nicotine addiction as well as memory function. 3)We use affinity purification with recombinant versions of the Ly-6/Neurotoxin (Lynx) proteins Lynx1 and Secreted Ly-6/uPAR-related protein 1 (SLURP-1) to show that Lynx proteins form complexes with α7 nAChRs in human brain extracts. Lynx1 has previously been shown to regulate the function of α7 nAChRs in heterologous expression systems and hamper associative memory and synaptic plasticity in mice by acting on nAChRs. Our results indicate that Lynx proteins may regulate α7 nAChR function in the human brain and affect not only cholinergic signalling, but also the effects of experimental drugs acting on the α7 nAChR.

Disclosures: **M.S. Thomsen:** F. Consulting Fees (e.g., advisory boards); Part time consultant for Bionomics Ltd. **E.N. Lyukmanova:** None. **M.A. Shulepko:** None. **J.D. Mikkelsen:** F. Consulting Fees (e.g., advisory boards); Advisory board member and part time consultant for Bionomics Ltd.

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.01/B97

Topic: B.02. Ligand-Gated Ion Channels

Support: CSIC Universidad de la Republica. Uruguay

Title: Direct actions of glycine on neurons of the mesencephalic trigeminal nucleus of the rat

Authors: V. SILVEIRA, F. R. MORALES, *I. POSE;
Facultad De Medicina, Montevideo, Uruguay

Abstract: Mesencephalic trigeminal (MesV) neurons are unique primary afferents because their cell bodies are located within the CNS and are contacted by numerous synapses containing different neurotransmitters. Thus, in addition to their sensory role, they may also function as

interneurons in networks that organize jaw movements. Recent available evidence indicates that MesV neurons of the rat are innervated by glycinergic fibers. The present study was conducted to examine the presence of glycinergic receptors on MesV neurons and the effects of glycine on their properties. Using specific antibodies we show that MesV neurons of the rat display immunoreactivity to the alpha 1, alpha 2 and beta subunits of the glycine receptor. The effects of glycine were studied in rat brainstem slices (P6-P20) in electrophysiological experiments with whole-cell recordings techniques. Glycine was bath or juxtacellularly applied. This neurotransmitter induced in all tested cells (35), a depolarization (2 to 8mV), a decrease in input resistance (20% to 25% reduction) and a decrease of the spike amplitude. These changes were accompanied, in some neurons, by an increase in excitability indicating that the depolarizing effect prevailed over the decrease in input resistance, whereas in others no obvious change in excitability was observed. Glycine exposure antagonized the excitatory effects of local glutamate application. The electrophysiological results suggest that glycine has complex actions on the excitability of MesV neurons, resulting excitatory in most cases but with the ability to reduce other excitatory actions. Our findings indicate that MesV neurons possess glycine receptors and are targets for glycine that may act on them as a neurotransmitter.

Disclosures: **V. Silveira:** None. **F.R. Morales:** None. **I. Pose:** None.

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.02/B98

Topic: B.02. Ligand-Gated Ion Channels

Support: Wellcome Trust Fellowship 101696/Z/13/Z

Title: Pathogenic mechanisms of glycine receptor antibodies in human disease

Authors: *S. J. CRISP[1], A. VINCENT[2], D. M. KULLMANN[1];
[1]Dept. of Clin. and Exptl. Epilepsy, Inst. of Neurology, Univ. Col. London, London, United Kingdom; [2]Nuffield Dept of Clin. Neurosciences, Univ. of Oxford, Oxford, United Kingdom

Abstract: Antibodies against glycine receptors (GlyRAbs) have been found in some patients with progressive encephalomyelitis with rigidity and myoclonus (PERM) (Carvajal-Gonzalez A et al, Brain 2014). PERM is a life-threatening acquired neurological syndrome characterized by axial and limb muscle stiffness and spasms, brainstem dysfunction such as oculomotor disturbance, stimulus-sensitive exaggerated startle responses (hyperekplexia), and autonomic

crises. However, patients with GlyRAbs who receive immunotherapy often make a substantial recovery, suggesting an antibody-mediated pathogenic process. Nonetheless, as with other neuropil autoantibodies, the relationship between the identified antibody and neurological disease remains conjectural. We are using a functional assay to explore the effects of patient IgG on glycinergic currents. Using whole-cell patch-clamp we have recorded spontaneous miniature inhibitory postsynaptic currents (mIPSCs) from motoneurons in rat dissociated spinal cord cultures. GABA and glycine are co-released at interneuron-motoneuron synapses, both contributing to mIPSCs (Jonas P et al, Science 1998). However, pharmacologically isolated glycinergic currents have a shorter decay time than GABAergic currents. We use this difference in time course to separate the two components, in the absence of pharmacological blockade, to quantify the contribution of glycinergic neurotransmission to mIPSCs recorded from motoneurons. We have compared the contribution to mIPSCs recorded from neurons incubated in patient or control IgG for up to 24h prior to recording. Our preliminary results show a decrease in the contribution of glycinergic current to the mIPSCs for neurons incubated in patient IgG compared with control IgG from 41% to 12% ($p<0.005$). A reduction in glycinergic neurotransmission in patients would be consistent with many of the clinical features of PERM. The electrophysiological findings presented here therefore provide strong evidence that the antibodies in these cases are pathogenic. The likely cellular mechanism is antibody crosslinking and internalization of the receptors as occurs in HEK293 cells expressing glycine receptors and incubated with patient sera (Carvajal-Gonzalez A et al, Brain 2014). We plan to explore whether this functional assay can also be used to detect antibodies in sera from patients suspected to have antibody-mediated deficiency in glycinergic neurotransmission, without an identified antigenic target. We will also examine whether IgG from patients with GlyRAbs who have atypical clinical presentations, such as encephalopathy, produce similar electrophysiological phenotypes.

Disclosures: **S.J. Crisp:** None. **A. Vincent:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Royalties and payments for antibody tests. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents for antibody tests, but no patent for glycine receptor antibody tests. **D.M. Kullmann:** None.

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.03/B99

Topic: B.02. Ligand-Gated Ion Channels

Support: DFG586

GSLS

Title: Functional ion channel properties are altered by a single amino acid exchange in the human glycine receptor α1

Authors: G. LANGLHOFER, P. BAUMANN, *C. VILLMANN;
Univ. Wuerzburg, Wuerzburg, Germany

Abstract: Communication between neurons of the central and peripheral nervous systems essentially relies on the balance of activation and inhibition at synaptic sites. Glycinergic neurotransmission is a key mediator of fast synaptic inhibition in adult spinal chord and brain stem of humans and rodents. Mutations of the postsynaptic glycine receptor (GlyR), its associated anchoring proteins or affected presynaptic glycine transporter 2 have been shown to underlie the rare human neuromotor disorder hyperekplexia, characterized by exaggerated startle reflexes and loss of postural control. Here we give a detailed description of the influence of a novel human mutation P366L, located within a proline-rich stretch in the intracellular loop connecting transmembrane domains 3 and 4 (TM3-4 loop) of the GlyRα1, on channel functionality. Using transfected HEK293 cells stained for the human α1 subunit, we demonstrate that the hyperekplexia-like symptoms observed from a patient suffering from this mutation are not due to disturbances in receptor biogenesis. In contrast, patch-clamp recordings unveil a significant reduction in glycine-induced maximum inward currents. The proline-rich domain 365PPPAPSKSP373 including the pathological mutation harbors a SH3 consensus sequence probably involved in binding of so far unidentified intracellular proteins. Functional analysis of constructs generated to incrementally destroy a possible secondary structure demonstrates a remarkable influence of the motif on desensitization properties of the human GlyRα1. The role of proteins involved in binding to this structure is still under investigation. Together these results provide a molecular explanation for impaired GlyR function finally leading to hyperekplexia-like symptoms observed in the patient carrying the mutation P366L.

Disclosures: G. Langlhofer: None. **P. Baumann:** None. **C. Villmann:** None.

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.04/B100

Topic: C.17. Drugs of Abuse and Addiction

Support: Fondecyt 3140194

NIH AA17875

dpi20140008

Title: Expression and subcellular localization of glycine receptor subunits and synaptic glycine transporter 2 in the nucleus accumbens of C57/BL6 mice

Authors: *B. FÖRSTERA[1], B. MUNOZ[1], K. STANIC[2], P. MURATH[1], L. G. AGUAYO[1]; [1]Dept. of Physiol., [2]Axon Guidance Lab., Univ. de Concepcion, Concepcion, Chile

Abstract: Introduction Alcohol abuse is a major health problem that affects millions of people worldwide, but the pharmacotherapeutic tools to assist behavioral intervention are limited and cause several undesirable side effects, which emphasizes the need for novel, mechanistically oriented therapies. Glycine gated chloride channels (GlyR) are potentiated by ethanol in mammalian neurons, resulting in a reversible increase in apparent affinity, and thus involved in the response of the CNS to alcohol. While the ethanol sensitive GlyR alpha1 subunit dominates in the spinal cord, GlyR in the brain are thought to be constituted mostly of alpha3 subunits, which are not affected by pharmacologically relevant concentrations of ethanol. Previous studies indicate the involvement of glycinergic currents in the nucleus accumbens (nAc) in alcohol consumption. After whole-cell patch-clamp recordings in dissociated neurons and acute slices of the nAc revealed strychnine sensitive glycine evoked currents and synaptic currents and showed that evoked currents where potentiated by low concentrations of ethanol, we investigated the expression and subcellular localization of GlyR subunits and the synaptic glycine transporter 2 (GlyT2) in the nAc. Results PCR indicates the predominant expression of the GlyR alpha1ins and alpha2A, as well as GlyT2, but also detects other GlyR subunit splice variants in the nAc. These findings are supported by western blot for GlyR alpha1 and GlyT2. Immunocyto- and -histochemistry in dissociated neurons and paraffin sections of the nAc using the same antibodies in combination with antibodies against GlyR pan-alpha, MAP2 and the vesicular inhibitory amino acid transporter (VIAAT) confirm the synaptic and extra-synaptic localization of these receptors in neurons of the nAc. Conclusion We identified GlyR alpha subunits and elucidated their expression and subcellular distribution, showing the synaptic and extra-synaptic presence of these receptors that enables them to support tonic and phasic currents in neurons of the nAc. These findings highlight GlyR as a target for innovative pharmacological approaches to treat alcoholism and other addiction-related behavior.

Disclosures: B. Förstera: None. **B. Munoz:** None. **K. Stanic:** None. **P. Murath:** None. **L.G. Aguayo:** None.

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.05/B101

Topic: B.02. Ligand-Gated Ion Channels

Title: Agonist and antagonist monoclonal antibodies with selectivity for ligand-gated glycine receptor isoforms

Authors: J. SIMARD[1], K. MICHELSEN[2], B. GRUBINSKA[1], Y. WANG[2], B. HALL[2], P. SHAFFER[2], *J. GINGRAS[1];
[1]Neurosciences, [2]Therapeut. Discovery, Amgen Inc., Cambridge, MA

Abstract: Ligand-gated ion channels, such as the glycine receptors (GlyRs) family, are targets of increasing interest for studies relevant to various neuronal diseases. Indeed, in the central nervous system, the most abundant GlyRs identified express either the alpha (α) 1 and/or the α3 subunits. Due to the high homology between the various GlyRα subunits, availability of selective tools necessary to better resolve their precise biological roles has been limiting. Here, we report the generation and characterization of multiple GlyRα3 antibodies. Selectivity against GlyRα1 was assessed by immunofluorescence, flowcytometry and surface plasmon resonance and their ability to modulate GlyRs activity investigated via an *in vitro* membrane potential dye assays on stable cell lines overexpressing human (hu) GlyRα1β or huGlyRα3β channels. We identified six functional dose-dependent mouse monoclonal antibodies with EC_{50} < 25nM and k_D values < 10nM. These include: three non-selective antagonist antibodies; one non-selective and two huGlyRα3β selective agonist antibodies. *In vitro*, the functional inhibition of these monoclonal antibodies can be blocked by pre-addition of a non-selective agonist/potentiator molecule, while their functional agonism can be prevented by pre-addition of strychnine, a known GlyRs antagonist. Together, this data demonstrates that ligand-gated ion channels functions can be modulated in a dose-dependent manner using high affinity antibodies and that, despite the high protein homology between the alpha subunits, selectivity is achievable within this receptor family.

Disclosures: **J. Simard:** A. Employment/Salary (full or part-time):; Amgen Inc. **K. Michelsen:** A. Employment/Salary (full or part-time):; Amgen Inc. **B. Grubinska:** A. Employment/Salary (full or part-time):; Amgen, Inc. **Y. Wang:** A. Employment/Salary (full or part-time):; Amgen.com. **B. Hall:** A. Employment/Salary (full or part-time):; Amgen Inc. **P. Shaffer:** A. Employment/Salary (full or part-time):; Amgen Inc. **J. Gingras:** A. Employment/Salary (full or part-time):; Amgen Inc..

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.06/B102

Topic: B.02. Ligand-Gated Ion Channels

Support: NSC 102-2320-B-039-038-MY3

MOST103-2320-B039-033

Title: Molecular mechanisms for zinc-induced allosteric potentiation of GlyR α1 receptors

Authors: C.-H. WANG[1], N. ZHOU[2], *D. WU[2];
[1]Grad. Inst. of Clin. Med. Science, China Med. Univ., Taichung, Taiwan; [2]China Med. University, Grad. Inst. of Clin. Med. Science,, Taichung, Taiwan

Abstract: The inhibitory glycine receptor (GlyR)-mediated fast synaptic transmission is involved in motor control and other physiological processes. Deficits in channel functions of GlyR α1 subunit are linked to human hyperekplexia. Previous studies and our recent findings have revealed that, when agonist binding and channel activation remains normal, the lack of positive zinc modulation on GlyR α1 is sufficient to affect normal synaptic transmission and leads to human hyperekplexia. We have also identified that the amino acid residue W170 is a new site that is involved in zinc-mediated potentiation on GlyR α1 and that W170 is located close to the previously identified zinc-binding sites, D194 and H215. However, it remains unknown how these residues constitute a molecular pathway that mediates the micro-structural changes around these residues and thereby transduces conformational changes from zinc binding to allosteric potentiation of GlyR. In the present study, we used site-directed mutagenesis to substitute W170 with hydrophobic, positive charged, negative charged, polar or no-polar residues and found that both the benzo ring and the hydrophobic properties of the Trp residue are required for maintaining zinc-mediated potentiation of GlyR α1. We further explored adjacent residues around 170 site and found that L195 mutations reduced the sensitivity of zinc-mediated potentiation. Single mutations of W170 into Cys not only abolished zinc-mediated potentiation, also dramatically reduced glycine-induced current responses of GlyR α1. Interestingly, introduction of a disulfide bond between W170 and L195 by double Cys mutations not only restored glycine-induced currents comparable to the wild type receptor, but also partially rescued zinc-mediated potentiation of GlyR α1. Furthermore, either W170C or I210C single mutations impaired the glycine-induced current response, which was, however, totally rescued by double mutations of W170C and I210C. Unlike the Cys cross-link of W170C/L195C, the cross-link of W170C/I210C did not rescue zinc-mediated potentiation on GlyR α1. Our results indicate that W170 can act as a hub between the zinc modulation pathway and the receptor activation

pathway. These findings also suggested that the connection between β8 and β10 strands is required for agonist-induced channel opening, whereas the connection between β8 and β9 strands is crucial for signal transduction from the allosteric modulation to the channel activation pathway.

Disclosures: **C. Wang:** None. **N. Zhou:** None. **D. Wu:** None.

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.07/B103

Topic: B.02. Ligand-Gated Ion Channels

Support: ARC Grant

NHMRC Grant

Title: Functional reconstitution of glycinergic synapses incorporating defined glycine receptor subunit combinations

Authors: ***Y. ZHANG**, C. L. DIXON, A. KERAMIDAS, J. W. LYNCH;
Queensland Brain Inst., Brisbane, Australia

Abstract: Glycine receptor (GlyR) chloride channels mediate fast inhibitory neurotransmission in the spinal cord and brainstem. Four GlyR subunits (α1-3, β) have been identified in humans, and their differential anatomical distributions result in a diversity of synaptic isoforms with unique physiological and pharmacological properties. To improve our understanding of these properties, we induced the formation of recombinant synapses between cultured spinal neurons and HEK293 cells expressing GlyR subunits of interest plus the synapse-promoting molecule, neuroligin-2A. In the heterosynapses thus formed, recombinant α1β and α3β GlyRs mediated fast decaying inhibitory postsynaptic currents (IPSCs) whereas α2β GlyRs mediated slow decaying IPSCs. These results are consistent with the fragmentary information available from native synapses and single channel kinetic studies. As β subunit incorporation is considered essential for localizing GlyRs at the synapse, we were surprized that α1-3 homomers supported robust IPSCs with β subunit incorporation accelerating IPSC rise and decay times in α2β and α3β heteromers only. Finally, heterosynapses incorporating α1D80Aβ and α1A52Sβ GlyRs exhibited accelerated IPSC decay rates closely resembling those recorded in native synapses from mutant mice homozygous for these mutations, providing an additional validation of our technique.

Glycinergic heterosynapses should prove useful for evaluating the effects of drugs, hereditary disease mutations or other interventions on defined GlyR subunit combinations under realistic synaptic activation conditions.

Disclosures: **Y. Zhang:** None. **C.L. Dixon:** None. **A. Keramidas:** None. **J.W. Lynch:** None.

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.08/B104

Topic: B.02. Ligand-Gated Ion Channels

Support: NHMRC APP1060707

ARC LP120100297

Title: New excitatory and inhibitory pharmacogenetic receptors based on the ivermectin-activated glycine receptor

Authors: ***J. W. LYNCH**[1], R. ISLAM[2];
[2]Queensland Brain Inst., [1]Univ. of Queensland, Brisbane, Australia

Abstract: The ability to control the electrical activity of defined neuronal populations *in vivo* is dramatically advancing our understanding of brain function. Optogenetic approaches, whereby light is used to activate anion or cation-selective channels, has proved a successful means of achieving both excitation and inhibition. However, as it requires surgically implanted light delivery probes, it may not be suited for activating populations of neurons distributed over a large parts of the brain. Pharmacogenetic approaches provide an alternate means of controlling the activity of widely distributed neuron populations. For example, neuronal silencing has been achieved with the invertebrate allatostatin-activated G protein-coupled receptor and excitation has been achieved with a synthetic M3 muscarinic receptor that is insensitive to acetylcholine but activated by the non-endogenous ligand, the clozapine-N-oxide. Alternate pharmacogenetic silencing methods based on ivermectin-activated glutamate or glycine receptor (GlyR) chloride channels have also been developed. We previously described a modified α1 GlyR incorporating the F207A mutation to eliminate glycine sensitivity and the A288G mutation to enhance ivermectin sensitivity to around 30 nM. Here we describe a new modified GlyR with significantly enhanced ivermectin sensitivity. This receptor, which incorporates the Y279F and A288G mutations, is reliably and potently activated by 3 nM ivermectin. We also developed an

excitatory GlyR, permeable to both sodium and calcium, that incorporates the A-1'E and A288G mutations. This produces potent neuronal depolarisation at 1 μM ivermectin. These tools may be useful for controlling neuronal activity *in vivo* and *in vitro*.

Disclosures: J.W. Lynch: None. **R. Islam:** None.

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.09/B105

Topic: B.02. Ligand-Gated Ion Channels

Title: Interaction of quinazolines with the E binding loop of the serotonin-type 3A (5-HT3A) receptor determined using double mutant cycling

Authors: *S. N. KHATRI[1,2], O. ALWASSIL[3], D. PHILIP[3], M. DUKAT[3], M. SCHULTE[2]; [1]Pharmacol. and toxicology, Univ. of Sci., Philadelphia, PA; [2]Dept. of Pharmaceut. Sci., Philadelphia Col. of Pharmacy, Univ. of Sci., Philadelphia, PA; [3]Dept. of Medicinal Chem., Sch. of Pharmacy, Virginia Commonwealth Univ., Richmond, VA

Abstract: The serotonin type 3A (5HT3A) receptors is a homomeric ligand gated ion channel receptor that has been targeted clinically for the treatment of chemotherapy induced emesis. More recently, 5-HT3 receptors have been shown to play a role in pain and cognition. They may also contribute to multiple neurological disorders including depression, epilepsy, anxiety, learning, and attention deficit disorders. In a recent study, analog of the 5-HT3R agonist mCPBG (3-CPG) enhanced the antinociceptive actions induced by clonidine in the mouse tail-flick assay without potentiating the side effects of clonidine. Based on molecular modeling, we identified two distinct interactions of 3-CPG with the 5-HT3AR. Our models suggest that the guanidine moiety of 3-CPG interacts with W183 and the aryl group interacts with Y143 of the 5HT3AR. Constrained quinazoline analogs of 3-CPG act as antagonists on the 5-HT3AR. During site directed mutagenesis analysis of the interaction of these compounds with W183 and Y153, we discovered that Y153A and W183F mutations converted these antagonists to partial agonists. To better understand the binding interactions of quinazoline analogs and possibly gain improved understanding of partial agonism on the 5-HT3R, we conducted a double mutant cycling study to determine if interactions were present at W183 and Y153 or whether observed changes in potency in these mutants were due to a functional effect of mutating these two residues. Our studies show that Y153 does not directly interact with the quinazoline analogs suggesting the observed changes in potency are due to an indirect or functional interaction.

Disclosures: **S.N. Khatri:** None. **O. Alwassil:** None. **D. Philip:** None. **M. Dukat:** None. **M. Schulte:** None.

Poster

669. Ligand Gated Ion Channels: Glycine and 5-Hydroxytryptamine Receptors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 669.10/B106

Topic: E.03. Behavioral Neuroendocrinology

Support: DA033358

Title: Effects of chronic caffeine exposure on rat brain serotonergic systems

Authors: *P. WILLIAMS;
Univ. of Colorado Boulder, Boulder, CO

Abstract: Chronic caffeine exposure during adolescence has been shown to induce persistent maladaptive anxiety-like behavioral responses in the adult rat. It is possible that these maladaptive responses are mediated by the serotonergic system. In this study, we investigated the effects of chronic adolescent caffeine exposure on the rat brain serotonin (5-hydroxytryptamine; 5-HT) system. Specifically, we analyzed serotonergic neuron activation in subregions of the dorsal raphe nucleus (DRN), a brainstem region with abundant serotonergic neurons. After a week of acclimatization, rats were randomly divided into four groups in a two-by-two experimental design. Two groups received chronic caffeine (CC) administration in drinking water (0.3 g/L) from postnatal day 28 to postnatal day 56 while the other two groups received drinking water (NC) alone during the same developmental time period. After 28 days of caffeine or control treatment and a 24-hour washout period, rats received an i.p. injection of either 30 mg/kg caffeine (C) or 0.9% sterile saline (S) vehicle, were then replaced in their home cages, and were euthanized 90 minutes following treatment. This was a 2 x 2 design with four treatment groups, NCS, NCC, CCS, and CCC. Using a double immunostaining technique we quantified the immunoreactivity for the acute activation marker c-Fos and tryptophan hydroxylase 2 (Tph2) as a marker of serotonergic neurons. NCC rats, relative to NCS and CCC groups, had higher activation of 5-HT neurons in the rostral DRD and caudal DRD, ventral part of the dorsal raphe nucleus (DRV), DRC, and DRI. These data are consistent with the hypothesis that the DRN is a key structure in promoting the adult pro-anxiety behavioral phenotype following adolescent caffeine exposure.

Disclosures: **P. Williams:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.01/B107

Topic: B.07. Synaptic Transmission

Support: NIH Grant R01MH104641

Title: Oxytocin receptor activation depolarizes spiny hilar interneurons and induces GABA release in the dentate gyrus of the rat hippocampus

Authors: *S. W. HARDEN[1,2,3], C. J. FRAZIER[1,2];
[1]Pharmacodynamics, [2]Neurosci., [3]Col. of Dent., Univ. of Florida, Gainesville, FL

Abstract: Aberrant central oxytocin (OXT) signaling is implicated in a variety of neurodevelopmental disorders, including autism spectrum disorder (ASD). However, relatively little is known about the physiology of central OXT signaling outside the hypothalamus, where OXT-synthesizing neurons reside. The dentate gyrus (DG) serves as the entry point of perforant path information flow into the hippocampus, and hilar interneurons (HINs) express OXT receptors (OXTRs) as demonstrated by in-situ hybridization and autoradiography studies. However, no reports exist which characterize the electrophysiological nature of OXTR activation in the DG, or its direct effect on HINs. In this study, we identify OXT-responsive HINs using cell-attached recordings and the selective OXTR agonist TGOT, and subsequently characterize these HINs electrophysiologically and morphologically utilizing whole cell patch-clamp recordings. Our results indicate that OXTR activation produces a strong but transient suprathreshold depolarization in a subset of HINs. Although the hilus has a heterogeneity of interneuron phenotypes, preliminary data indicate OXT-responsive neurons are GABAergic spiny interneurons. Interestingly, OXTR responsive HINs also often display intrinsic peri-threshold oscillations, as well as an unreported form of choline-independent, persistent firing behavior that can be terminated with strong transient depolarization. Further, both of these phenomena appear to be modulated by OXTR activation. Continued investigation of OXT-responsive HINs and their effect on the excitatory pathways they modulate will provide insight into the role of OXT as a neuromodulator of hippocampal neurotransmission, and may aid in the development of pharmacological therapeutics for ASD and other neurodevelopmental disorders.

Disclosures: S.W. Harden: None. **C.J. Frazier:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.02/B108

Topic: B.07. Synaptic Transmission

Support: AG047652

Title: Anatomical and functional expression of channelrhodopsin-2 (ChR2) in the basal forebrain and thalamus of the Vglut2-ChR2-eYFP BAC optogenetic mouse

Authors: *D. W. DUBOIS, D. A. MURCHISON, K. S. MONTGOMERY, A. S. FINCHER, A. H. MAHNKE, W. H. GRIFFITH;
Dept. of Neurosci. and Exptl. Therapeut., Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX

Abstract: Disruption of synaptic function is a major contributor to cognitive decline during aging. Our lab has focused on understanding the relationship between altered synaptic physiology and age-related cognitive impairment in the rodent basal forebrain (BF). Our long term goal is to reverse cognitive decline by maintaining youthful synapses across aging. The BF has a complex synaptic circuitry featuring multimodal inputs onto cholinergic, GABAergic, glutamatergic and peptidergic neurons, as well as cortically projecting neurons originating in the BF. Previous data from our lab suggests that the postsynaptic current densities of AMPA glutamate receptors in the rat BF increased with age (Jasek and Griffith, Neuroscience, 82:1179-94, 1998). Because some glutamatergic BF neurons are thought to express vesicular glutamate transporter 2 (Vglut2) (Colom et al., Synapse, 58:151-164, 2005), we tested the idea that synaptic glutamate release from Vglut2 neuron terminals may contribute to synaptic plasticity during aging. To examine the network connectivity of glutamatergic synaptic transmission within the BF, we used light-evoked synaptic currents from Vglut2-ChR2 (H134R)-eYFP BAC optogenetic mice (1-4 mo). We combined whole-cell patch clamp electrophysiology and LED-evoked light stimulation (470 nm) in brain slices and in a reduced synaptic preparation in these optogenetic mice. Postsynaptic neurons were identified by biocytin filling and immunohistochemistry, or by single cell RT-PCR. Despite previous research showing widespread Vglut2 immunoreactivity in the BF, confocal microscopy of BF slices revealed that the vast majority of eYFP fluorescence was localized in the medial septum with limited fluorescence in the vertical limb of the diagonal band and essentially no eYFP positive neurons found in the horizontal limb. Using prolonged (5-10s) light stimulation, no glutamatergic synaptic transmission was detected in neurons from BF slices (0/46 cells). In contrast, confocal images from the thalamus displayed a robust number of eYFP positive cells, and when patch-clamped, these neurons (21/27) displayed inward ChR2 currents in response to brief (5 ms) light

stimulation. scRT-PCR confirmed that 100% of eYFP fluorescent thalamic neurons were negative for both ChAT and GAD (n=11) and were presumably glutamatergic. The Vglut2-ChR2 (H134R)-eYFP BAC optogenetic mouse works well in some brain areas, but it may not be ideal for studying BF glutamatergic transmission. Future experiments will seek to confirm this finding in the BF reduced synaptic preparation and to identify age-related functional and expressional changes in the Vglut2-eYFP-ChR2 construct.

Disclosures: D.W. Dubois: None. **D.A. Murchison:** None. **K.S. Montgomery:** None. **A.S. Fincher:** None. **A.H. Mahnke:** None. **W.H. Griffith:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.03/B109

Topic: B.07. Synaptic Transmission

Support: CIHR MOP-79277

Title: Brain-derived estradiol controls hunger state-dependent plasticity of GABA synapses

Authors: *J. GARDNER GREGORY, E. R. HAWKEN, S. ANGELIS, E. C. DUMONT; Ctr. for Neurosci., Queens Univ., Kingston, ON, Canada

Abstract: The oval bed nucleus of the stria-terminalis (ovBNST) is one of the most sexually dimorphic regions of the brain, containing androgen, estrogen and progestin receptors. It is a region critical in controlling both feeding and anxiety-related behaviours. We showed that GABA inhibitory post-synaptic current (IPSC) in the ovBNST is a neurophysiological mechanism regulating these behavioural phenomena. Interestingly, estradiol (E2) has both anxiogenic and anorectic effects, but whether and how estradiol in the ovBNST regulate these behavioural phenomena is unknown. Therefore, we examined the neurophysiological and behavioural effects of E2 in the ovBNST. We used both naïve male and female Long-Evans rats and conducted whole cell patch clamp electrophysiology in the ovBNST measuring GABA IPSC's. Bath application of E2 potentiated GABA IPSC's both in male and female rats, although the effect varied with the estrous cycle in females. E2 potentiated GABA through estrogen receptor alpha (ERα). Furthermore, we observe that a low frequency stimulation (LFS) protocol caused a long-term potentiation (LTP) of GABA synapses that was dependent on both the production of E2 and the activation of ERα. E2 acted post-synaptically and ultimately interfered with a pre-synaptic tonic inhibition of GABA IPSCs by endocannabinoids. Finally, 24 hour food

restriction had the ability to reduce LFS induced GABA LTP and unmasked a long-term depression of GABA synapses. The neurophysiological effects of acute food restriction on GABA IPSC's are mostly reversed by either refeeding the rat or the activation of estrogen receptors. Therefore, estradiol is critical in GABA plasticity within the ovBNST and appears to be essential in the regions regulation of feeding behaviours.

Disclosures: J. Gardner Gregory: None. **E.R. Hawken:** None. **S. Angelis:** None. **E.C. Dumont:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.04/B110

Topic: B.07. Synaptic Transmission

Support: CIHR MOP-79277

Title: Impaired gaba plasticity at ovbnst synapses predicts compulsive drinking in cfr-induced schedule-induced polydipsia

Authors: *S. ANGELIS, J. GARDNER GREGORY, E. R. HAWKEN, M. H. NAUGHTON, C. P. NORMANDEAU, E. C. DUMONT;
Queens Univ., Kingston, ON, Canada

Abstract: Schedule-Induced Polydipsia (SIP) is an animal model of compulsivity resulting in an adjunctive drinking behavior in food restricted animals. The bed nucleus of the stria terminalis (BNST) is involved in anxiety, food intake, and goal directed behaviors and may thus be an important modulator of compulsive behavior. *In vivo* electrophysiology stimulation activity in the BNST indicate that rats who develop compulsive drinking had higher neuronal firing rates of BNST neurons compared to controls and rats. The aim of the current research was to investigate the excitatory and inhibitory neurophysiological properties in the ovBNST underlying SIP. Rats were food restricted during the 21 days of SIP training, only having a 1 hour access to food daily. During SIP training rats were individually placed in an operant conditioning. Food pellets were delivered every minute for a total of 120 minutes in the food magazine and water bottles were weighed before and after each session. To be considered a compulsive drinker, rats must consume ≥15 ml of water for a minimum of 3 consecutive days during the session. At the end of 21 days of training, the rats were divided into two groups: SIP (compulsive drinkers) and SIP-RES (non-compulsive drinkers). At the end of training, rats were either continued to be food

restricted overnight or given ad libitum food. The rats were euthanized and their brains harvested so that *in vitro* whole cell patch-clamp electrophysiology in the ovBNST can be conducted. Glutamatergic and GABAergic synaptic signalling were investigated through AMPA/NMDA ratios and low frequency stimulation respectively. There were no differences in AMPA/NMDA ratios across all groups. There were no differences observed in GABA plasticity between the non-refeed rats in the SIP and SIP-RES conditions, however there were significant differences in the refeed conditions such that SIP rat neurons exhibited a long-term depression in contrast to the long-term potentiation observed in the SIP-RES rats. These findings indicate that an impaired GABA plasticity in the ovBNST may be a biomarker for the development of schedule-induced polydipsia.

Disclosures: **S. Angelis:** None. **J. Gardner Gregory:** None. **E.R. Hawken:** None. **M.H. Naughton:** None. **C.P. Normandeau:** None. **E.C. Dumont:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.05/B111

Topic: B.07. Synaptic Transmission

Support: NIH/NIA Grant AG047652

Title: Cell type differences in light-evoked GABAergic synaptic transmission in basal forebrain neurons from VGAT-ChR2-eYFP optogenetic mice

Authors: *K. S. MONTGOMERY[1], D. W. DUBOIS[2], D. A. MURCHISON[2], A. S. FINCHER[2], A. H. MAHNKE[2], U. H. WINZER-SERHAN[2], U. H. WINZER-SERHAN[2], W. H. GRIFFITH[2];
[1]Neurosci. & Exptl. Therapeut., [2]Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: We have previously shown that GABAergic synaptic transmission in the rat basal forebrain (BF) is altered with age and cognitive status (Griffith et al., J.Neurophysiol., 111:273-286, 2014). Here we examine the network connectivity of GABAergic synaptic transmission within the BF using light-evoked synaptic currents in identified neurons from VGAT-ChR2 (H134R)-eYFP BAC optogenetic mice. Our long term goals are to examine the anatomical and functional expression of channelrhodopsin-2 (ChR2) over the lifespan and to determine the properties of inhibitory synaptic transmission across a backdrop of aging and cognitive decline.

Present experiments utilize mice of either sex (1-4 mo), while future experiments will utilize mice from our aging colonies. We are using whole-cell patch clamp electrophysiology and LED light-evoked excitation (470 nm) in brain slices and a "reduced synaptic preparation," consisting of acutely dissociated neurons where synaptic boutons remain attached, for direct measurements of synaptic release. Postsynaptic neurons were identified as GABAergic or cholinergic by biocytin filling and immunohistochemistry, or by single cell RT-PCR. Postsynaptic functional expression of ChR2 was determined in GABAergic neurons by generating input/output curves for light-evoked ChR2 inward currents (1-200 mA, with 100 mA=1.76 mW/mm^2). With synaptic currents blocked, cells displayed a sigmoidal curve with amplitude dependent on ChR2 expression. With intact synaptic transmission, brief light pulses (2-5 ms) evoked synchronous IPSCs in the majority of GABAergic neurons (8/10). Whereas in 12 of 17 non-GABAergic neurons (cholinergic or others), asynchronous synaptic release was observed. Paired-pulse (PP) stimulation resulted in PP depression (n=6). During prolonged light stimulation (5-10 s), GABAergic neurons displayed a small 10% increase in mean IPSC frequency (n=5). In contrast, non-GABAergic neurons showed a substantial increase in mean IPSC frequency during light stimulation (1400%, n=8). These results highlight the cell type specific differences in the local BF inhibitory network. In order to explore these differences further, experiments are underway using confocal microscopy to stimulate synaptic boutons. These parameters will be examined across aging. Ultimately, a better understanding of excitatory and inhibitory synaptic mechanisms in young and aged animals is a necessary first step in elucidating the cellular mechanisms susceptible to change as a function of age, cognitive status and/or drug treatment.

Disclosures: K.S. Montgomery: None. **D.W. DuBois:** None. **D.A. Murchison:** None. **A.S. Fincher:** None. **A.H. Mahnke:** None. **U.H. Winzer-Serhan:** None. **U.H. Winzer-Serhan:** None. **W.H. Griffith:** None.

Poster

670. GABAergic Synapses

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.06/B112

Topic: B.07. Synaptic Transmission

Support: PAPIIT Grant IN212313

Title: Insulin modulates GABAA-mediated tonic currents in the prefrontal cortex

Authors: *S. L. HERNANDEZ[1], S. TRUJEQUE-RAMOS[2], S. MIHAILESCU[2];
[2]Fisiologia, [1]Facultad de Med., Mexico, Mexico

Abstract: Extrasynaptic GABA receptors are high-affinity GABAA receptors located away from the synaptic sites and activated by ambient GABA concentrations. They are responsible for the tonic GABAA-mediated inhibition that regulates the excitability of cells and neural networks. These tonic GABAA receptor-mediated currents are found in different brain areas showing cell type-specific differences in magnitude and pharmacology. It has been suggested that GABAA extrasynaptic receptors are probably the most important sites of action of diverse drugs like benzodiazepines and general anesthetics, since they are activated by very low GABA concentrations and their activation causes tonic inhibition. Recent studies, have shown that insulin regulates the expression of extrasynaptic GABAA receptors in the hippocampus, causing permanent changes in the neuronal circuits. This effect could explain the alterations of cognitive processes associated to changes in insulin signaling previously reported. Another structure that possess insulin receptors and is involved in cognitive functions is the prefrontal cortex. In this work, we used patch clamp recordings in brain slices to examine the effect of insulin on the tonic GABAA receptor-mediated currents in the prefrontal cortex (layers 5-6). We found that insulin (10-500 nM) increased the tonic current recorded from pyramidal cells. Our data suggests that insulin increases the trafficking of extrasynaptic GABAA receptors to the membrane and that these receptors contain alpha-5 and delta subunits. Also, our results demonstrate that this effect of insulin decreases the excitability of pyramidal neurons.

Disclosures: S.L. Hernandez: None. **S. Trujeque-Ramos:** None. **S. Mihailescu:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.07/C1

Topic: B.07. Synaptic Transmission

Support: NIH Grant MH078823

NIH Grant MH104506

NIH Grant MH099658

Title: Inhibitory co-transmission with glutamate in hippocampal primary neurons

Authors: *C. A. BURLESON, H.-J. SHU, S. MENNERICK;
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Abstract: Chemical transmission is more complex than previously realized. Several examples of cells utilizing multiple classical neurotransmitters have now been discovered, including glutamate and γ-amino butyrate (GABA) co-transmission. In hippocampus, co-transmission of glutamate and GABA has been controversial. Here, we provide direct evidence of inhibitory co-transmission in glutamatergic hippocampal pyramidal neurons. Utilizing cultured single neuron microislands, we confirmed that in addition to relatively large AMPA receptor-mediated autaptic currents, a subset of glutamatergic neurons exhibit a much smaller $GABA_A$ receptor ($GABA_AR$) mediated current only detectable in the presence of AMPA and NMDA receptor antagonists. We show that this "occult" inhibitory postsynaptic current (IPSC) is abolished by tetrodotoxin (TTX), and is dependent on extracellular Ca^{2+}, suggesting the transmitter responsible is released from axon terminals and is packaged into vesicles. The occult IPSCs were potentiated by several $GABA_AR$ positive allosteric modulators, including the endogenous neurosteroid allopregnanolone (272 ± 67% of control), and were abolished by the $GABA_AR$ antagonist gabazine. While GABA is the most appealing candidate neurostransmitter, occult IPSCs had significantly faster decay kinetics compared to bona fide IPSCs in GABAergic interneurons. Loading GABA into presynaptic vesicles through endocytosis in neurons with an occult current produced IPSCs with decay kinetics slower than endogenous occult currents (272 ± 24% of control). However, incubation in 10-20 μM GABA for 1-2 hours potentiated occult IPSCs (250 ± 81% of control) without changing the decay kinetics. Bona fide IPSCs were not significantly altered by GABA incubation. These contradictory effects of different GABA loading protocols on occult IPSCs could be explained by the expression of different $GABA_ARs$ at occult synapses and glutamatergic synapses; GABA loading through endocytosis does not discriminate between terminals and may activate $GABA_ARs$ distinct from those present in occult IPSC synapses. Despite the ambiguity in responsible co-transmitter, we demonstrate that single glutamatergic hippocampal primary neurons can also transmit inhibitory signals through $GABA_ARs$ that are modifiable by endogenous neuromodulators.

Disclosures: C.A. Burleson: None. **H. Shu:** None. **S. Mennerick:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.08/C2

Topic: B.07. Synaptic Transmission

Support: CONACyT CB (MA)

Title: Nicotine reduces inhibitory synaptic currents in mouse layer 5 prefrontal cortex

Authors: *R. D. CUEVAS OLGUIN[1], E. ESQUIVEL-RENDÓN[2], O. IBAÑEZ-SANDOVAL[3], H. ARIAS[4], M. ATZORI[2];
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Abstract: Nicotine use alters numerous brain functions including working memory. Synaptic transmission in the infragranular layers controls the output from the prefrontal cortex, a critical area for executive function and working memory. We investigated the effects of nicotine on synaptic transmission of the output layers of the medial prefrontal cortex (mPFC) of C57BL/6 mice in a brain slice preparation, using whole-cell patch-clamp recording. Synaptic currents were evoked onto visual identified neurons in layer 5 by electrical stimulation of local afferents in a brain slice preparation. We found that bath applications of nicotine (0.3 microM) induce a reversible decrease of mixed excitatory and inhibitory synaptic currents. In order to determine whether inhibitory (vs. excitatory) synaptic currents were affected by nicotine, we repeated a similar experiment in the presence of the blockers of the α-amino-3-hydroxyl-5-methyl-4-isoxazol-propionate receptors (AMPAR) 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 microM), and of the N-methyl-D aspartate receptors (NMDAR) kynurenic acid (2 mM). Similar to the previous experiment, bath-applied nicotine markedly reduced the amplitude of gamma amino butyric acid type A receptors (GABAAR) mediated currents, by approximately 50%.

Disclosures: R.D. Cuevas Olguin: None. **E. Esquivel-Rendón:** None. **O. Ibañez-Sandoval:** None. **H. Arias:** None. **M. Atzori:** None.

Poster

670. GABAergic Synapses

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Program#/Poster#: 670.09/C3

Topic: B.07. Synaptic Transmission

Support: CAPES

CNPq

FAPERGS

Title: Changes in inhibitory synaptic transmission in glutaric acidemia type I

Authors: *M. E. CALCAGNOTTO[1], L. MEIER[1], M. VENDRAMIN PASQUETTI[1], B. JUNGES[1], M. GANZELLA[1], S. LOUREIRO[1], A. UMPIERREZ AMARAL[1], D. M. KOELLER[2], S. I. GOODMAN[3], M. WOONTNER[3], M. WAJNER[1], D. GOMES DE SOUZA[1]; [1]Biochem., UFRGS, Porto Alegre, Brazil; [2]Pediatrics, Oregon Hlth. and Sci. Univ., Portland, OR; [3]Pediatrics, Univ. of Colorado Denver, Aurora, CO

Abstract: Glutaric academia type I (GAI) is an inherited glutaryl-CoA dehydrogenase (GCDH) deficiency characterized by progressive dystonia, neurological deficit, macrocephaly and recurrent seizures. An increased level of organic acids in the cerebral cortex and striatum in GAI seems to inhibit glutamic acid decarboxylase (GAD) activity that may lead to decreased GABA levels in the brain parenchyma. This decreased inhibition could contribute to seizure activity. In this work, we used an animal model of GAI, the GCDH knockout mice under lysine overload diet ($Gcdh^{-/-}$-Lys), to evaluate the cortical GABAergic synaptic transmission using biochemical and electrophysiological approaches. All animal procedures were performed according the local Ethics Commission (CEUA/HCPA approval #140270-120472). *Neurochemical parameters:* Cortical synaptosomes were used to evaluate GABA release by HPLC and GAD immunocontent by Western blot. GAD activity was determined by HPLC with specific GAD inhibitor. Electrophysiology: Spontaneous and miniatures inhibitory postsynaptic currents (sIPSC and mIPSC) were recorded in layer V cortical pyramidal neurons from brain slices of $Gcdh^{-/-}$-Lys mice using visualized IR-DIC whole cell voltage patch-clamp technique (h.p = 0mV). To isolate GABA currents, slices were perfused with nACSF containing CNQX/APV, and TTX (for mIPSC). The same biochemical and electrophysiological experiments were performed in age-matched (P30-P45) $Gcdh^{-/-}$ mice under normal diet ($Gcdh^{-/-}$-nd), and $Gcdh^{+/+}$ mice under either normal or lysine overload diet (controls). Our results show that GABA release before and after depolarization was low in $Gcdh^{-/-}$-nd (n=8) but lower $Gcdh^{-/-}$-Lys mice (n=11) when compared with controls (n=11) ($p<0.05$). This significant reduction in GABA release was associated with a decreased in cortical GAD immunocontent and activity from $Gcdh^{-/-}$-Lys (n=7, 13) when compared with $Gcdh^{-/-}$-nd mice (n=8, 14) and controls (n=15, 18) ($p<0.05$). Moreover, cortical pyramidal cells from $Gcdh^{-/-}$-Lys mice exhibited decrease in sIPSC frequency (2.4 ± 0.4Hz, n=5) when compare to $Gcdh^{-/-}$-nd mice (5.4 ± 0.9Hz, n=5) and controls (5.5 ± 0.9HZ, n=5), ($p<0.05$). Frequency of mIPSC onto cortical pyramidal cells from $Gcdh^{-/-}$-Lys mice was also decreased (1.4 ± 0.1Hz, n=6) when compare to $Gcdh^{-/-}$-nd mice (4.6 ± 0.4Hz, n=6) and controls (4.1 ± 0.1HZ, n=6), ($p<0.05$). No difference was found in sIPSC and mIPSC amplitude, decay time constant or rise time. Our data suggest a reduction in cortical inhibition in $Gcdh^{-/-}$-Lys mice probable caused by GAD dysfunction with consequent decrease in GABA levels that may contribute to the epileptogenesis in GAI.

Disclosures: M.E. Calcagnotto: None. **L. Meier:** None. **M. Vendramin Pasquetti:** None. **B. Junges:** None. **M. Ganzella:** None. **S. Loureiro:** None. **A. Umpierrez Amaral:** None. **D. M.**

Koeller: None. **S. I. Goodman:** None. **M. Woontner:** None. **M. Wajner:** None. **D. Gomes de Souza:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.10/C4

Topic: B.07. Synaptic Transmission

Support: American FOundation for Pharmaceutical Education

The Epilepsy Foundation

Center on Aging at the University of Utah

Interdepartmental Program in Neuroscience

Title: 5-HT6 receptor ligands modulate seizure thresholds and inhibitory synaptic transmission in the dentate gyrus

Authors: *G. J. REMIGIO[1], G. W. SAUNDERS[2], P. J. WEST[3,2];
[2]Anticonvulsant Drug Develop. Program, [3]Dept. of Pharmacol. & Toxicology, [1]Univ. of Utah, Salt Lake City, UT

Abstract: 5-HT6 receptor antagonists exhibit procognitive, and possibly anticonvulsant, effects. However, their effects on memory in animal models of Alzheimer's disease (AD), and in multiple acute seizure tests, are lacking. Moreover, how 5-HT6 receptor antagonists produce their procognitive and anticonvulsant effects remains unclear. Thus, we hypothesized that 5-HT6 receptor activation enhances inhibitory neurotransmission in the DG, and that 5-HT6 receptor antagonists improve DG-dependent memory in a transgenic model of AD while having paradoxically anticonvulsant activity in naïve mice. To test the acute anticonvulsant effects of 5-HT6 antagonists, drug-induced changes in minimal clonic seizure thresholds were assessed in naïve mice. To test the effects of 5-HT6 receptor activation on neurotransmission, coronal brain slices containing dorsal hippocampus were prepared from naïve rats. Field potential recordings and patch-clamp electrophysiology were used to test the effects of a 5-HT6 receptor agonist WAY-208466 (1μM) on baseline field excitatory postsynaptic potentials (fEPSPs), as well as evoked and spontaneous inhibitory postsynaptic currents (eIPSCs, sIPSCs). To assess spatial memory in a transgenic model of AD, male J20 mice and their non-transgenic littermates were injected with the 5-HT6 receptor antagonist SB-399885 or methyl cellulose and tested in the

metric task, which relies on spatial memory. Drug effects were compared with the Fisher exact test, Student's t-test, or ANOVA. SB-399885 (10mg/kg, 30 min, i.p.) was proconvulsant in C57Bl6 mice but had no effect in CF1 mice. Another antagonist, SB-271046 (10mg/kg, 30 min, i.p.), had no effect on seizures in either species. Additionally, the 5-HT6 receptor agonist WAY-208466 significantly decreased the amplitude of fEPSPs in the DG in brain slices from naïve rats. WAY-208466 also increased the amplitude of eIPSC onto DG granule cells. Finally, preliminary data suggest that SB-399885 (10mg/kg, 30 min, i.p.) reverses spatial memory impairments in J20 mice; further testing is underway. In conclusion, the 5-HT6 receptor antagonist SB-399885 exhibited proconvulsant effects in the minimal clonic seizure test in C57BL6 mice. The 5-HT6 receptor agonist WAY-208466 attenuated the amplitude of fEPSPs and increased the amplitude of eIPSCs onto DG GCs. Additionally, preliminary data suggests that SB-399885 may reverse memory impairments in a transgenic model of AD. Together, these results suggest that 5-HT6 receptor antagonists, which are in clinical trials in AD, have complex effects on synaptic transmission in the DG and may affect seizure liability and cognition.

Disclosures: G.J. Remigio: None. **G.W. Saunders:** None. **P.J. West:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.11/C5

Topic: C.07. Epilepsy

Support: NIH/NINDS

Title: Ultrastructure of basket cell-to-granule cell synapses in a rat model of temporal lobe epilepsy

Authors: *P. BUCKMASTER, K. THIND, R. YAMAWAKI;
Dept Comparative Med., Stanford Univ., Stanford, CA

Abstract: Reduced inhibition of hippocampal granule cells might contribute to the generation of seizures in temporal lobe epilepsy, so it is important to identify underlying mechanisms. Abnormally low frequencies of miniature inhibitory postsynaptic currents in granule cells are a common finding in different rat models of temporal lobe epilepsy. Previously, recordings of monosynaptically coupled neurons revealed impaired transmission at basket cell-to-granule cell synapses in epileptic rats, which was hypothesized to be attributable to fewer docked vesicles. To test this hypothesis, in the present study randomly selected axo-somatic symmetric synapses

were evaluated in the granule cell layer of control and epileptic rats 2-6 months after pilocarpine-induced status epilepticus (4 rats/group). Synaptic boutons (10/rat) were reconstructed 3-dimensionally from serial 35-nm-thick sections. Electron micrographs were shot at 36,000X and printed at a final magnification of 90,000X so that docked vesicles could be identified by their contact with the presynaptic membrane. Boutons were reconstructed from an average of 39 ultrathin sections (range 17-82 sections/bouton). Preliminary results suggest the following average control values: 0.4 μm^3 bouton volume, 21% bouton volume as mitochondria, 1.5 synapses/bouton, 0.12 μm^2 synapse area, 1000 vesicles/bouton, 12 docked vesicles/bouton, and 63 vesicles within 50 nm of a synapse. Values from epileptic rats that were significantly different from controls include twice as many vesicles/bouton (2042, p=0.007, t test), 2.4-times more docked vesicles/bouton (29, p=0.04), and 1.8-times more vesicles within 50 nm of a synapse (115, p=0.048). These findings reveal more, not fewer, docked vesicles in epileptic rats, suggesting that in epileptic animals basket cell-to-granule cell synaptic transmission might be dysfunctional at a step following vesicle docking, such as vesicle priming or vesicle fusion.

Disclosures: P. Buckmaster: None. **K. Thind:** None. **R. Yamawaki:** None.

Poster

670. GABAergic Synapses

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Program#/Poster#: 670.12/C6

Topic: B.07. Synaptic Transmission

Support: DC009635/DC/NIDCD NIH HHS/United States

DC12557/DC/NIDCD NIH HHS/United States

R00 DC009635/DC/NIDCD NIH HHS/United States

R01 DC012557/DC/NIDCD NIH HHS/United States

Title: Oxytocinergic modulation and plasticity in cortical and subcortical circuits

Authors: *J. SCHIAVO, R. FROEMKE;
New York Univ., New York, NY

Abstract: The neuropeptide oxytocin regulates a variety of social behaviors ranging from sociosexual to parental behavior (Insel and Young, 2001; Dulac et al., 2014; Nakajima et al., 2014). Recently, it was shown that oxytocin administration in the left auditory cortex enables

pup retrieval, a behavior characteristic of maternal females, in virgin female mice by modulating cortical responses to pup calls (Marlin et al., 2015). Oxytocin rapidly reduced inhibitory post-synaptic currents (IPSCs) *in vitro* and reduced call-evoked IPSCs *in vivo* in the left auditory cortex while gradually enhancing excitatory post-synaptic currents (EPSCs) and temporal precision of spiking. This provided evidence that oxytocin may disinhibit cortical circuits in a similar manner to acetylcholine to increase the salience of social stimuli. In the present study, we aim to further understand the role of oxytocin in cortical disinhibition and subsequent induction of long-term plasticity. Oxytocin binds to a single isoform of a G-protein coupled receptor (Gimpl & Fahrenholz, 2001), but it still remains unknown what type of neurons express this receptor and the response properties of these populations following oxytocin receptor activation. Using *in vitro* whole-cell recordings, we examined the disinhibitory effect of oxytocin in brain regions containing a high density of oxytocin receptor labeling, such as the piriform cortex and paraventricular nucleus, to determine if there are general principles by which oxytocin modulates diverse neural circuits. In addition, this study examined the ability of oxytocin to induce a form of long term potentiation in the auditory cortex by measuring the effects of oxytocin on evoked spike probability and the ratio of excitation to inhibition (E:I ratio). We confirmed the finding that oxytocin induces a rapid reduction in IPSC peak amplitude in layer 5 pyramidal neurons of the auditory cortex following bath application of oxytocin and endogenous activation of oxytocin release via optogenetic stimulation of PVN terminals. Subsequently, E:I ratio increases and is maintained for an extended period of time following cessation of endogenous and exogenous stimulation. Similar effects in the PVN and piriform cortex were observed. In addition, we found that oxytocin results in an increase in evoked spike probability and an increase in EPSP slope during oxytocin receptor activation (via both optogenetic stimulation and bath application) and is maintained for 6-10 minutes following cessation. Our study provides a potential mechanism by which oxytocin may induce long-term neural plasticity in the brain.

Disclosures: **J. Schiavo:** None. **R. Froemke:** None.

Poster

670. GABAergic Synapses

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 670.13/C7

Topic: D.08. Pain

Support: KAKENHI 24500461

Title: Developmental change in the modulation by oxytocin of synaptic transmission in rat spinal substantia gelatinosa neurons

Authors: *C.-Y. JIANG, T. FUJITA, L. ZHU, C. WANG, T. YU, R. HIRAO, E. KUMAMOTO;
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Abstract: Although there is much evidence indicating that a posterior pituitary hormone oxytocin is involved in antinociception in the spinal dorsal horn, this action has not yet been examined thoroughly. We previously reported that bath-applied oxytocin produces an inward current at a holding potential of -70 mV without a change in glutamatergic spontaneous excitatory transmission while enhancing GABAergic and glycinergic spontaneous inhibitory transmission in adult (6-8 weeks old) male rat spinal lamina II (substantia gelatinosa; SG) neurons. The SG neurons play a pivotal role in regulating nociceptive transmission from the periphery. These oxytocin responses were mimicked by an oxytocin-receptor agonist TGOT and inhibited by its antagonist. The inward current was resistant to a voltage-gated Na^+-channel blocker tetrodotoxin, Ca^{2+}-free and non-NMDA receptor antagonist; the inhibitory transmission enhancements were depressed by tetrodotoxin. In spinal superficial dorsal horn neurons of young (2-4 weeks old) male rats, on the other hand, it has been reported that TGOT increases the spontaneous release of L-glutamate on GABAergic interneurons, resulting in spontaneous GABA release enhancement. Glycinergic spontaneous inhibitory transmission was unaffected by TGOT. In order to reveal a detail of the developmental change in oxytocin actions in the spinal dorsal horn, the present study examined the effect of oxytocin (0.5 μM) on synaptic transmission in SG neurons of young (< 3 weeks old) rat spinal cord slices by using the conventional blind whole-cell patch-clamp technique. Moreover, we addressed whether there is a difference between male and female rats in synaptic modulation by oxytocin. Superfusing oxytocin increased the frequency of spontaneous excitatory postsynaptic current recorded from young male rat SG neurons, as reported previously. On the other hand, oxytocin enhanced not only GABAergic but also glycinergic spontaneous inhibitory transmission in young as well as adult rat SG neurons. With respect to a change in holding currents at -70 mV, oxytocin produced not only inward but also outward currents. Female adult and young SG neurons exhibited inward current and glutamatergic spontaneous excitatory transmission enhancement, respectively. These results indicate that synaptic modulation by oxytocin in SG neurons alters with development in a similar manner in both male and female rats and that not only inhibitory transmission enhancement but also membrane hyperpolarization produced by oxytocin may contribute to its antinociceptive effect in young rats.

Disclosures: **C. Jiang:** None. **T. Fujita:** None. **L. Zhu:** None. **C. Wang:** None. **T. Yu:** None. **R. Hirao:** None. **E. Kumamoto:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.01/C8

Topic: B.08. Synaptic Plasticity

Support: NFR Grant

Title: Regulation of Arc protein by SUMOylation during LTP in the adult dentate gyrus *in vivo*

Authors: *S. S. PATIL, R. R. NAIR, C. BRAMAHAM;
Neurosicence, Inst. of biomedicine, Bergen, Norway

Abstract: Arc protein is posited as a master regulator of long-term synaptic plasticity in the mammalian brain. The requirement for Arc synthesis in both long-term potentiation (LTP) and long-term depression (LTD) implies functional versatility of Arc protein. Biochemically, Arc is flexible modular protein with many binding partners. Post-translational conjugation by SUMO (small ubiquitin protein-like modifier) is important for regulation of protein-protein interactions in cells. Arc is SUMOylated *in vitro* but the role of endogenous Arc SUMOylation is largely unknown. Here, we examined Arc SUMOylation following LTP induction by brief bursts of high-frequency stimulation applied to medial perforant input to the dentate gyrus of adult anesthetized rats. Sustained Arc synthesis over a period of hours is required to stabilize this LTP. Using co-immunoprecipitation assays performed in dentate gyrus lysate, we obtained evidence for rapid SUMO1-ylation of newly synthesized Arc during the maintenance phase of LTP. SUMOylated Arc was highly enriched in the synaptoneurosomal fraction relative to whole lysate. The results demonstrate rapid SUMOylation of newly synthesized Arc during LTP *in vivo*.

Disclosures: S.S. Patil: None. **R.R. Nair:** None. **C. Bramaham:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.02/C9

Topic: B.08. Synaptic Plasticity

Support: NIH Grant F32 MH100752

Title: A potential role for postsynaptic synaptotagmins in LTP

Authors: *D. WU, T. BACAJ, W. MORISHITA, D. GOSWAMI, R. MALENKA, T. SÜDHOF; Stanford Univ., Stanford, CA

Abstract: Experience-dependent synaptic plasticity in the mammalian brain underlies fundamental neurological processes such as learning and memory. One prominent form of synaptic plasticity is NMDA receptor-dependent long-term potentiation (LTP), which occurs when Ca2+ influx into the postsynaptic spine following NMDA receptor activation triggers a series of downstream events, eventually leading to exocytosis of AMPA receptor-containing vesicles and insertion of AMPA receptors into the postsynaptic plasma membrane. The molecular mechanisms of vesicle fusion has been studied in detail at the presynaptic active zone. The core machinery of membrane fusion are the SNARE proteins: syntaxin, synaptobrevin/VAMP, and SNAP. Synaptic vesicle fusion, which is dependent on Ca2+ entry into the presynaptic terminal, additionally requires the action of Ca2+-sensing proteins, synaptotagmins, in conjunction with the cofactor complexin. Thus far, SNARE proteins and complexin have also been identified as obligatory molecular components of AMPA receptor exocytosis postsynaptically. However, a putative Ca2+ sensor for AMPAR exocytosis has yet to be found. Here we explore the potential role of synaptotagmins in Schaffer collateral-CA1 LTP in the hippocampus. Since identifying a synaptotagmin function for postsynaptic vesicular traffic would provide significant progress in our understanding of LTP but represents a difficult challenge, a multipronged approach will be necessary to achieve this. Given the requirement of complexins for LTP, we feel that it is likely that a synaptotagmin will be involved.

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Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

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Program#/Poster#: 671.03/C10

Topic: B.08. Synaptic Plasticity

Support: Conacyt: CB166241

Title: LPS-induced neuroinflammation alters synaptic plasticity at the mossy fiber - CA3 synapse

Authors: ***A. M. AVILA**, G. HERRERA-LOPEZ, C. TECUATL-TOLAMA, E. GALVAN; Cinvestav, Cuidad de Mexico, Mexico

Abstract: Lipopolysaccharide (LPS) is a pathogen-associated molecular pattern derived from gram-negative bacteria and recognized by Toll-like receptor (TLR)-4, the principal stimulator of inflammation. In the hippocampal formation, activation of TLR4 is implicated in the process of neuro-inflammation that also causes an unbalance of the synaptic transmission. Here we sought to determine synaptic alterations at the mossy fibers - CA3 synapses following endotoxemia induced with systemic injection of LPS (1 mg/kg). Extracellular recordings were performed in the stratum lucidum of area CA3b of hippocampal slices 24 hours after the immunological challenge. Although baseline synaptic transmission was unaffected in the LPS-treated animals, paired pulse facilitation showed a decrease (control MF PPF = 2.4 ± 0.3; LPS-treated animal 1.3 ± 0.1; ISI = 60 ms.). When high frequency stimulation (100 Hz, repeated 3 times at 10 sec interval) was applied at the mossy fibers, induction of MF LTP was blunted in the LPS-treated animals (MF PTP = 450 ± 70%; MF fEPSP at 90 min post HFS 230 ± 10%; in the LPS-treated animals PTP = 230 ± 30%; MF fEPSP at 90 min post HFS 111% ± 20%). The MF-origin of the evoked responses was systematically verified in all the experiments by applying DCG-IV 1 μM (MF fEPSP depression >95%). Immunohistochemistry experiments revealed a reduction in the expression of GAD67 on the LPS-treated slices. Lastly, western blots obtained from microdissected regions of stratum lucidum showed a stronger phosphorylation of Akt, TrkB and CamKII in LPS-treated mice. Our results suggest that the acute and systemic LPS exposure alters different signaling pathways involved in the induction of MF LTP.

Disclosures: **A.M. Avila:** None. **G. Herrera-Lopez:** None. **C. Tecuatl-Tolama:** None. **E. Galvan:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Program#/Poster#: 671.04/C11

Topic: B.08. Synaptic Plasticity

Support: National Natural Science Foundation of China #39970241

Title: Roles of 5-HT3a receptors in hippocampal long-term synaptic plasticity and learning

Authors: *Y. HUANG, Y. YU, D. CAO, R. HAO, Y. QI, H. XU, X. LIU, N. LU;
Fudan Univ., Shanghai, China

Abstract: The 5-hydroxytryptamine type-3a receptor (5-HT3aR) as the only ligand-gated ion channel in serotonin receptor family is known to involve in schizophrenia and anxiety. However, the physiological role and mechanisms of 5-HT3aRs in synaptic plasticity and memory remains unclear. Here we show that in the CA1 region of mouse hippocampus, pharmacological blockade (ondansetron, 25nM) or genetic deletion of 5-HT3aR specifically impaired theta burst or 200Hz high frequency stimulation induced long-term potentiation (LTP) and LFS induced long-term depression (LTD), while basal glutamatergic neurotransmission and the metabotropic glutamate receptor dependent LTD were not affected by 5-HT3aR perturbations. In addition, 5-HT3aRs disruption inhibited AMPA receptors (AMPARs) internalization, without altering basal surface levels of AMPARs. However, the deletion of 5-HT3aRs did not lead to loss of synapses and structural alteration of dendritic spines. The concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus were not affected by the deletion of 5-HT3aRs either. Moreover, behavioral studies showed that 5-HT3aR knock-out mice exhibited impaired hippocampus-dependent learning and memory in morris water maze, but had no change in motor coordination and balance. These results reveal a direct role of 5-HT3aR in hippocampal synaptic plasticity and spatial memory, which may have implications for understanding the cognitive impairments in psychiatric disorders.

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Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: CNPq Brazil 161239/2011-0

Title: Chronic nicotine reverses memory impairments and attenuates hippocampal-medial prefrontal cortex synaptic plasticity dysfunctions in the streptozotocin model of sporadic Alzheimer's disease

Authors: *I. M. ESTEVES[1], C. LOPES-AGUIAR[2], R. N. RUGGIERO[2], M. T. ROSSIGNOLI[2], L. KANDRATAVICIUS[2], R. N. ROMCY-PEREIRA[3], J. P. LEITE[2];

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Abstract: Severe abnormalities in brain glucose/energy metabolism and insulin signaling have been documented to play an important role in early stage of AD (esAD) pathology. An intracerebroventricular administration (icv) of subdiabetogenic doses of streptozotocin (STZ) in rats can induce an insulin-resistant brain state associated to cholinergic dysfunctions, memory impairments and formation of beta amyloid plaques, which make it a suitable experimental model of esAD. Usually, studies with this model have focused upon measure cognitive impairment and molecular dysfunction. there are no studies that have addressed the effect that chronic treatment of nicotine has on CA1-mPFC synaptic plasticity in STZ rat model. Here, Wistar rats received bilateral microinjection of STZ and were submitted to 20 days of nicotine treatment. After 2 days of withdraw, the subjects were submitted to open field and object recognition tests to access locomotion and evaluate recognition memory, respectively. On day 28, rodents were anesthetized with urethane for electrophysiological tests. Twisted bipolar electrode was used to stimulate CA1 with paired-pulse protocol (two monophasic square-pulses; duration=0.2ms; inter-stimulus interval=80ms; rate=0.05Hz) and basal field post-synaptic potentials (fPSP1) and facilitated responses (fPSP2) were recorded by a monopolar electrode in the mPFC. After 30min of baseline, high frequency stimulation (HFS) was applied to induce long-term potentiation (LTP) and additional four hours of electrophysiological recordings was performed. HFS consisted of two series of 10 trains (50 pulses at 250Hz every 10s), 10min apart. Our results indicate that STZ produced a significant decrease in the induction and maintenance of LTP. The nicotine treatment attenuates the STZ-induced LTP dysfunction in the CA1-mPFC pathway. Besides, PPF shows that the shortterm synaptic plasticity, impaired by STZ injection, was reversed by nicotine treatment. These results are associated to behavioral data, since animals just injected with STZ also displayed significant deficits in novel object recognition task and general decrease exploratory behavior in the open field test. Nicotine treatment reversed the deficits in recognition memory but maintained the reduced exploratory behavior. We suggest that the brain cholinergic system, which plays an important role in cognition function and LTP, is affected inSTZ injected animals and a chronic treatment with nicotine can attenuate the STZ-induced synaptic plasticity and behavioral dysfunctions.

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Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.06/C13

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS21184

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Texas Emerging Technologies Fund

Title: The developmental onset age of hippocampal LTP in mice

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Abstract: Long-term potentiation (LTP), a persistent strengthening of synapses after patterned stimulation, has been widely accepted as a cellular substrate of learning and memory. In order to test whether dendritic spines are required for enduring LTP that lasts more than 3 hours, we investigated the developmental onset age for enduring LTP in rats and mice. Previously, we found that the onset age for enduring LTP induced by theta-burst stimulation (TBS) is postnatal day (P)12 in hippocampal area CA1 of Long Evans rats (Cao and Harris, 2012). In addition, we found that prior to P20, test pulses delivered even as slowly as 1 pulse/5 minutes produced synaptic depression. The test pulse-induced depression is frequency-dependent, as higher frequency test pulses induced more depression than lower frequencies. Before the LTP onset age, TBS reversed the test pulse-induced depression in P8-11, but no potentiation was produced above the original naïve response. An additional episode of TBS delivered 30-120 minutes after the first TBS produced enduring LTP at P10-11, but not at younger ages in rats (P8-9). We further examined the developmental profiles of test pulse-induced depression and onset age for enduring LTP in C57BL/6 mice. Similar to rats, test pulses induced synaptic depression in P9-12 mice. However, the test pulse-induced depression in mice was dependent on the number instead of the frequency of test pulses. After ~ 120 pulses at 0.2Hz or 0.5Hz, the test pulse-induced depression reached a plateau. Similar to rats, test pulse-induced depression attenuated as the animals became older, and disappeared in young adult mice (P60). Unlike in rats, the developmental onset for enduring LTP in mice was much later, as enduring LTP could not be reliably induced with TBS until ~P35. Furthermore, unlike in rats, an additional episode of TBS did not produce enduring LTP in mice right before the onset age (P28-33). Preliminary results from our lab showed P12 to be the onset age of mature dendritic spines in rat hippocampal area CA1, suggesting they are necessary for enduring LTP. Prior work shows dendritic spines occur

by P15 (Nikonenko et al., 2013) in C57BL/6 mouse hippocampal area CA1, suggesting dendritic spines may be necessary, but not sufficient for enduring LTP in the mouse.

Disclosures: **G. Cao:** None. **K.M. Harris:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

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GRSNC Fellowship

CIHR Studentship

Title: Metaplastic regulation of CA1 Schaffer collateral pathway plasticity by Hebbian mGluR1a-mediated plasticity at excitatory synapses onto somatostatin-expressing interneurons

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Abstract: Cortical GABAergic interneurons represent a highly diverse neuronal type that regulates and synchronizes neural network activity. In particular, interneurons in the hippocampal CA1 oriens/alveus (O/A-INs) area provide feedback dendritic inhibition to local pyramidal cells and express somatostatin (SOM). Under relevant afferent stimulation patterns, they undergo long-term potentiation (LTP) of their excitatory synaptic inputs through multiple induction and expression mechanisms. However, the cell type specificity of these different forms of LTP and their specific contribution to the dynamic regulation of the CA1 network remain unclear. Here we addressed these questions using cell-specific transgenic mice lines expressing enhanced yellow fluorescent protein (EYFP) under the control of SOM (SOM-Cre-EYFP) or parvalbumin (PV-Cre-EYFP). Immunofluorescence detection in hippocampal CA1 revealed that SOM-expressing interneuron (SOM-INs) somas were located mostly in stratum oriens and

alveus whereas PV-expressing interneuron (PV-INs) somas were mainly found in and around the pyramidal cell layer, indicating a specific labeling of mostly non-overlapping interneuron populations. We then used whole cell recordings from SOM- and PV-INs and found that pairing theta burst stimulation (TBS) delivered at the O/A border with postsynaptic depolarization induced a Hebbian LTP in SOM-INs. Bath application of 40 μM LY367385 prevented the induction of LTP, revealing a metabotropic glutamate receptor type 1a (mGluR1a) dependence. However, TBS failed to induce LTP in PV-INs, showing that mGluR1a-mediated Hebbian LTP occurs in a cell type-specific fashion in CA1 interneurons. We then used field recordings from transgenic mice expressing archaerhodopsin Arch3 selectively in SOM-INs to assess the role of LTP in SOM-INs in the regulation of synaptic plasticity in the Schaffer collateral pathway of pyramidal cells. We found that high frequency stimulation (HFS) applied in the stratum radiatum elicited LTP in pyramidal cells, and this LTP was up-regulated by a prior conditioning TBS in O/A. LTP facilitation was prevented by light-induced hyperpolarization of SOM-INs during TBS, or by application of the mGluR1a antagonist LY36785, indicating a necessity for mGluR1a and SOM-INs activation. These results uncover that mGluR1a-dependent LTP in SOM-INs performs an activity-dependent metaplastic control on hippocampal CA1 microcircuits in a cell-specific fashion. Our findings provide new insights on the contribution of interneuron synaptic plasticity in the regulation of the hippocampal network activity and mnemonic processes.

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Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Program#/Poster#: 671.08/C15

Topic: B.08. Synaptic Plasticity

Support: DGAPA-PAPIIT UNAM: IN210515

Title: Contribution of M-currents to the effects of neurotrophins on long-term potentiation in sympathetic ganglion

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Abstract: We have previously shown that brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) acting on TrkB or p75NTR, and TrkA receptors, differentially regulate

long-term potentiation (gLTP) in rat sympathetic ganglion. Additionally, we have characterized the presence of neurotrophin receptors TrkA, TrkB and p75NTR in intact superior cervical ganglia of adult Wistar rats. Taking into account that BDNF and NGF acting on Trk receptors produce opposite effects on neuronal excitability, depending on the activation or inhibition of KCNQ/M-currents, either in sympathetic or in central neurons. Herein, we asked if this current could play a role as a possible effector in the previously reported effects of BDNF and NGF on gLTP. To characterize the possible involvement of KCNQ channels in the phenomenon of gLTP, we applied a train of stimulus (40 Hz, 3 sec.) in the cervical sympathetic trunk, and recorded the post-synaptic responses (compound action potentials) in presence of XE991 (a Kv7/KCNQ channel antagonist). We found that blockade of KCNQ channels with XE991 [1 μM] mimicked the effect of NGF on gLTP, producing a significant reduction in gLTP, LTP decay time and LTP extent decreased ~55% of control values ($p< 0.04$). Furthermore, we found that the stimulatory effect of BDNF on gLTP was abolished by co-application of XE991, thus in this experimental condition both LTP decay time and LTP extent were similar to control gLTP values ($p >0.1$). Our data suggest that the M-currents are involved in the modulation of the gLTP by neurotrophins in sympathetic ganglion, however, the contribution of other effectors to this modulation can not be excluded. Therefore, we propose that BDNF acting on TrkB or p75NTR receptors would activate Kv7 channels, whereas NGF through TrkA activation would inhibit these channels, resulting in a differential regulation of synaptic plasticity in sympathetic ganglionic synapses.

Disclosures: **F.R. Cifuentes:** None. **E.R. Arias:** None. **M.A. Morales:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: Dutch NWO-ALW grant (VENI: 863.12.017) to Geeske van Woerden

Title: Functional requirement of hippocampal CaMKII in long-term potentiation

Authors: ***M. J. KOOL**, N. Z. BORGESIUS, M. R. HOJJATI, G. M. VAN WOERDEN, Y. ELGERSMA;
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Abstract: With a highly unique network architecture the CA3 region of the hippocampus functions critically in higher-order spatial processing. Specifically, long-term potentiation (LTP) of both Schaffer-collateral (CA3-CA1) and associational/commissural (CA3-CA3) pathways has been widely proposed as candidate mechanisms for hippocampus-dependent spatial processing. At the molecular level, the calcium/calmodulin-dependent protein kinase II (CaMKII) is among the most abundant proteins in the hippocampus, and widely demonstrated to function critically in regulating the postsynaptic induction of LTP at the hippocampal Schaffer-collateral CA3-CA1 synapse. However, little is known about the independent presynaptic and postsynaptic requirements for CaMKII during LTP at the CA3 synapses. Therefore, we investigated the requirement of α- and βCaMKII for Schaffer collateral (CA3-CA1) and associational/commissural (CA3-CA3) LTP using a selective genetic ablation of *Camk2a* and *Camk2b* in the CA3 neurons of the hippocampus. In contrast to the conventional *Camk2a* and *Camk2b* global knockout mice, CA3-*Camk2a* and CA3-*Camk2b* KO mice showed robust and stable CA3-CA1 LTP. In addition, LTP of the associational/commissural pathway between CA3 neurons was intact in both CA3-*Camk2a* and CA3-*Camk2b* KO mice. However, when both α- and βCaMKII were deleted simultaneously in CA3 neurons, LTP at the associational/commissural pathway between CA3 neurons was impaired while LTP at the Schaffer-collateral pathway was unaffected. Taken together, αCaMKII and βCaMKII appear to function in a highly specific manner for regulating LTP within hippocampal circuits and the highly homologous α and β isoforms of CaMKII are redundant as shown by impaired LTP at the CA3-CA3 synapse only upon deletion of both isoforms simultaneously.

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Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

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Topic: B.08. Synaptic Plasticity

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Title: AMPA receptor dynamics and LTP regulated by a cyclin protein

Authors: *E. CHO[1,2], D.-H. KIM[3], Y.-N. HUR[1], D. J. WHITCOMB[3,4], P. REGAN[3,4], J.-H. HONG[1], H. KIM[1], Y. SUH[5], K. CHO[3,4], M. PARK[1,2];
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Abstract: Cyclin Y (CCNY) is a member of the cyclin protein family, known to regulate cell division in proliferating cells. Interestingly, CCNY is expressed in neurons that do not undergo cell division. Here, we report that CCNY negatively regulates long-term potentiation (LTP) of synaptic strength through inhibition of AMPA receptor trafficking. CCNY is enriched in postsynaptic fractions from rat forebrain and is localized adjacent to postsynaptic sites in dendritic spines. We found that during LTP-inducing stimulation, CCNY inhibits AMPA receptor exocytosis in dendritic spines. Furthermore, knockdown of CCNY enhances LTP in hippocampal slices. Taken together, our findings demonstrate that CCNY inhibits plasticity-induced AMPA receptor delivery to synapses and thereby blocks LTP, identifying a novel function for CCNY in post-mitotic cells.

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Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: Canadian Institute of Health Research

CREATE training program in Biophotonics from the Natural Sciences and Engineering Research Council of Canada

Title: Optical Imaging of miniature synaptic Ca^{2+}-transients to monitor synaptic potentiation

Authors: *G. NADEAU, T. WIESNER, M. LEMIEUX, P. DE KONINCK;
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Abstract: Classical measurements of synaptic plasticity have involved electrophysiological methods which provide high sensitivity for detecting small changes in synaptic strength. However, this approach does not provide much information about the location of the synapses that undergo plastic changes. Because synaptic plasticity can be synapse-specific, having the ability to monitor changes in synaptic strength at individual synapses is important in order to enable simultaneously monitoring of local molecular mechanisms associated with the plasticity. New fluorescent tools developed in the last decades allow to directly visualize synaptic activity, signaling, and remodeling at individual synapses. In this study, we are using optical imaging of a genetically-encoded Ca2+ sensor, GCaMP6f, to record miniature synaptic Ca2+-transients (MSCTs) in cultured rat hippocampal neurons. For these experiments, we perform video-microscopy on neurons perfused with external solution lacking Mg2+ and containing Tetrodotoxin (TTX). We have observed highly localized and transient increases of intracellular Ca2+ in dendritic compartments and spines. To test whether these MSCTs can be potentiated, we have measured them before and after a 5 min stimulation known to induce plasticity in cultured neurons (0Mg2+/Glycine, cLTP). A lasting increase in the frequency and amplitude of MSCTs, for at least an hour, arose from this stimulation protocol. We are thus investigating the molecular mechanisms of this plasticity. The MSCTs are mostly mediated by NMDA receptors, since they are almost totally blocked by the selective antagonist to the receptor, AP5. Moreover, addition of AP5 only during the cLTP stimulation blocks the MSCT plasticity. It thus appears that both the MSCTs and their plasticity are NMDA receptor-dependent. Interestingly, the MSCTs and their plasticity are not blocked by the AMPA receptor antagonists NBQX, pointing to possible changes in NMDA receptor content, post-synaptic Ca2+ signaling, or neurotransmitter release. To test theses hypotheses, we are combining Ca2+ imaging with electrophysiological measurements of NMDA receptor currents, imaging of other pre and postsynaptic components, and various pharmacological treatments to identify the molecular mechanisms responsible for the MSCT plasticity. Our approach might provide new knowledge on the diversity of molecular processes that support synaptic potentiation.

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Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS051187

Title: TARP γ8 and GSG1L function in GluA1 and GluA4 subunit-specific synaptic delivery of AMPARs in classical conditioning

Authors: *J. KEIFER, N. K. TIWARI, Z. ZHENG;
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Abstract: Appropriately timed interactions of AMPARs with specific scaffolding proteins and kinases determines their subcellular localization and function. Previously, we developed a two-stage model of AMPAR trafficking for classical conditioning in an *in vitro* model of eyeblink classical conditioning in which GluA1-containing AMPAR synaptic delivery occurs early in conditioning followed later by synthesis and delivery of GluA4 subunits that replace GluA1 and supports conditioning (Zheng et al., J Neurophysiol. 108: 101, 2012). We also showed that GluA1 and GluA4 subunits with associated kinases are sequentially delivered to synapses by the common scaffolding protein SAP97 (Zheng & Keifer, JBC 289: 10540, 2014). Since there is a common interacting protein, how subunit-specific synaptic delivery of native AMPARs is achieved during this form of learning was the goal of this study. Using an isolated preparation of the pons from turtles, an auditory nerve conditioned stimulus (CS) paired with a trigeminal nerve unconditioned stimulus (US) generates a neural discharge characteristic of a fictive "blink" learned response in the abducens nerve. High-resolution proteomics analysis has revealed a diversity of AMPAR-associated proteins but the function of these auxiliary proteins in AMPAR trafficking is just beginning to be revealed. Using Western blots, we found that protein levels for TARP γ8 and GSG1L are regulated in conditioning with GSG1L being significantly increased during conditioning. Co-immunoprecipitation (co-IP) studies show that TARP γ8 interacts with GluA1 early in conditioning after 15 min and one pairing session (C1 or 25 min) but this is undetectable after two pairing sessions (C2 or 80 min). In contrast, GluA4 interacts with GSG1L later in conditioning reaching its peak at C2. Unexpectedly, GluA4 also interacts with TARP γ8 at C2. Therefore, TARP γ8 appears to chaperone GluA1-containing AMPARs during early stages of conditioning while GSG1L chaperones later arriving GluA4 subunits. Based on these findings, we hypothesize that subunit-specific trafficking of AMPARs during conditioning is achieved through not only the timing of their interactions with auxiliary proteins but also by selected protein assemblies marking each subunit.

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Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS081786

Title: Metabolic processes driving long term potentiation

Authors: ***P. MIRANDA**[1], H.-A. PARK[1], C. PEQUIGNOT[2], S. SACCHETTI[1], H. LI[1], K. ALAVIAN[3], H. IMAMURA[4], H. NOJI[5], J. SHEPHERD[6], A. CHAVES[7], R. S. ZUKIN[7], E. A. JONAS[1];
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Abstract: Long-term potentiation (LTP) and depression (LTD) are the mechanisms by which neurons modulate their inherent synaptic plasticity and support the storage and recovery of memories in the mammalian brain. The ability to potentiate a synapse declines significantly in neurodegenerative disorders and is related to deficiencies in LTP. In addition to deficiencies in synaptic plasticity, degenerating neurons display acute and chronic mitochondrial dysfunction, suggesting that dysregulated mitochondria play a hand in synaptic dysfunction in addition to their known role in apoptotic death. Our previous work shows that the anti-apoptotic protein Bcl-xL not only prevents somatic cell death, but it also potentiates long-term synaptic responses. Here, we show that Bcl-xL is responsible for dramatic changes of ATP levels at synapse-specific sites in hippocampal neurons. Using fluorescent imaging of an ATP-FRET construct in living cells, we find that LTP induction in control neurons causes a short decrease in ATP levels followed by a persistent long term increase in ATP production. This suggests that after high frequency or intense synaptic stimulation, neurons may become metabolically more efficient; oxygen consumption rates during LTP are now being performed to confirm or refute the proposed change in efficiency. The long-term increase in ATP levels of LTP-stimulated synapses is blocked when we inhibit Bcl-xL. Bcl-xL inhibition also prevents a long-term increase in surface glutamate receptor insertion. In hippocampal slice recordings, inhibition of Bcl-xL greatly impairs early stage LTP and prevents late stage LTP. We suggest that long term changes

in mitochondrial efficiency brought on by activity-dependent translocation of Bcl-xL to mitochondria are required for LTP. Our studies shed light on the role of changes in mitochondrial metabolic efficiency in the acute induction and long term maintenance of learning and memory storage. If such mitochondrial changes fail to occur, synaptic dysfunction associated with neurodegeneration may ensue. Our study places mitochondria and Bcl-xL at the center of synaptic metabolism and synaptic plasticity.

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Poster

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Support: NTP(T32 GM007507)

NIH/NINDS RO1NS065067

Title: The epigenetic modulation of aberrant synaptic plasticity in Tuberous Sclerosis Complex

Authors: *T. BASU, K. J. O'RIORDAN, W. POTTER, N. KHAN, A. ROOPRA;
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Abstract: Tuberous Sclerosis Complex (TSC) is an autosomal dominant, multi-system spectrum disorder that affects approximately 1 in 6,000 people. The disorder is characterized by the formation of benign growths that most commonly develop in the brain, kidney, heart, lungs, eyes and skin (Curatolo, P., 2002; Crino, P.B. 2006 & 2013). Patients with TSC display developmental delays, cognitive defects, autism and epilepsy. This disease is caused by a loss of function mutation in either the TSC1 or TSC2 genes, resulting in the disinhibition of mammalian Target of Rapamycin (mTOR), a key regulator of cell survival and proliferation, protein synthesis and metabolism (van Slegtenhorst, 1997; Tang, S.J., 2002; Hou, L., 2004). Current treatment involves the modulation of hyperactive mTOR activity, but chronic mTOR inhibition may have adverse effects on patient health (Schindler, C.E., 2014; Carracedo, A., 2008; Kusne, Y., 2014). There is a need to find a TSC specific treatment that does not present with harmful side effects. Acute hippocampal slices from adult male heterozygous TSC2 mutant mice

(TSC2+/-) display abnormal long term potentiation (LTP) and long term depression (LTD). A 1X theta burst stimulation elicits short term potentiation in adult male wild type (WT) hippocampi, but this paradigm induces LTP in hippocampi obtained from gender and age matched TSC2+/- mice. In contrast to adult male WT mice, the TSC2+/- mice display a rapamycin insensitive form of metabotropic glutamate receptor (mGluR) mediated LTD. We have shown that adult TSC2+/- mice have increased Erk1/2 signaling that bypasses the canonical mTOR signaling cascade to drive atypical synaptic plasticity (Potter, W., 2013). A whole genome expression analysis of cortical samples resected from human TSC and non-TSC patients suggests that chromatin alterations around key genes encoding components of the Mek/Erk signaling pathway appear to drive the plasticity alteration upon loss of a TSC allele. We find that the modification of chromatin structure through the pharmacological inhibition of histone deacetylation and demethylation attenuates Erk1/2 signaling and restores normal LTP and LTD in adult male TSC2+/- mice. This study is the first to describe epigenetic mechanisms influencing synaptic plasticity alterations in TSC and can unearth a novel therapeutic option for TSC patients.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NUS Research Scholarship

National Medical Research Council, Singapore

Title: Modulation of synaptic plasticity and associativity in hippocampal CA2 pyramidal neurons by substance P

Authors: ***A. DASGUPTA**[1], M. HAKIM[2], S. SREEDHARAN[3];
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Abstract: Mammalian brain is well known for its ‘plasticity’ i.e. the ability of an experience-generated neural activity to alter the strength and efficacy of its neural circuits and has been

widely studied in hippocampal CA1 area. Recently area CA2 is in the limelight as this small region which is largely ignored over many years, is now found to play a pivotal role in the formation of social and emotional memory. This area is innervated by supramammilary (SuM) axonal fibres that are abundant in Substance P (SP), a tachykinin neuropeptide which acts both as a neurotransmitter and neuromodulator of pain in nervous system. These innervations could possibly modulate the excitability of hippocampal neurons and its theta rhythm. Although, SP is released from the presynaptic vesicles of the SuM axons which terminate into the hippocampal CA2 subfield, not much information is available regarding the role of it in CA2 and is yet to be investigated. In this study, we found that, bath application of substance P leads to protein synthesis and N-methyl-D-aspartate (NMDA) receptor dependent plasticity and late associativity such as synaptic tagging and capture only in CA2 but not in CA1 where SP is mostly known to have a memory enhancing effect. Overall, our study indicates that SP can be a critical regulator of plasticity and associativity in CA2 pyramidal neurons and could be a potential mediator for emotional and social memory formation. Furthermore, substance P which coexists with most of the cholinergic neurons, could facilitate the cholinergic transmission in area CA2, rich in both nicotinic and muscarinic cholinergic innervations and play an important role in memory formation. Thus, it can not be ruled out that SP could be a major player in the formation and enhancement of social memory in CA2 and can be an important target for Alzheimer's and other forms of dementia that lead to cognitive decline due to defects in cholinergic transmission.

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Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.16/C23

Topic: B.08. Synaptic Plasticity

Support: China NSF Grant 31271155

Title: Long-term potentiation of excitatory synaptic transmission from ipsilateral pericentral canal to motoneurons of neonatal rat spinal cord slices

Authors: H. SONG[1], L. ZHANG[1], W. QIN[1], Y.-H. SHI[1], *M.-Y. WANG[2];
[1]Cell Electrophysiol Lab., [2]Wannan Med. Col., Wuhu, Anhui 241002, China

Abstract: Our previous study has shown that the excitatory postsynaptic potentials (EPSPs) evoked by ipsilateral pericentral canal (iPCC) stimulation, iPCC-EPSPs, recorded in

motoneurons (MNs) may be mediated by heterogeneous transmitter-receptor mechanisms in addition to glutamate-NMDA and AMPA receptors. To explore the possible synaptic plasticity of excitatory synaptic transmission from ipsilateral pericentral canal, a presumed site of "central pattern generator" of motor control, to MNs in spinal cord, the intracellular recordings were performed in MNs of spinal cord slices isolated from neonatal rats (8-14 days old) and iPCC-EPSPs were mainly induced by electrical stimulation of iPCC. After the tetanic electrical stimulation (100 Hz, 50 pulses/train, duration 0.3~1.0 ms, 6 trains, main interval 10 seconds, 20~100 V) on iPCC in 24 MNs with iPCC-EPSPs, the amplitude of iPCC-EPSPs was enhanced to more than 120% of the baseline and the enlargement lasted longer than 30 min in 8 MNs, which could be referred to as long-term potentiation (LTP, iPCC-LTP). During iPCC-LTP, the area under curve and maximum left slope of iPCC-EPSPs were increased comparable to amplitude change of iPCC-EPSPs, while with the varied duration and latency changes compared to control. The analyses of apparent receptor kinetics (Acta Physiol Sin 2014, 66: 129) of iPCC-EPSPs during iPCC-LTP showed that apparent association rate constant (K1) increased, apparent dissociation rate constant (K2) and apparent equilibrium dissociation constant (KT) decreased only in 4 MNs. It was also observed that the induction of iPCC-LTP was facilitated by pretreatment of bicuculline (30 μM) and strychnine (1 μM), but the maintenance of iPCC-LTP was nullified by APV (30 μM) and DNQX (1 μM). However, the estimated parameters of cell electrophysiological properties of recorded MNs were not significantly altered. These preliminary data show that the MN's activities are modulated by the iPCC intraspinal activation, and the possible long-term synaptic plasticity is induced in those pathways, which may be considered as a candidate of neural basis underlying the "central pattern generator" of motor control.

Disclosures: **H. Song:** None. **L. Zhang:** None. **W. Qin:** None. **Y. Shi:** None. **M. Wang:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.17/C24

Topic: B.08. Synaptic Plasticity

Support: NSF Grant IOS1048556

Title: Septal facilitation of normal and LTP electrophysiological responses in the rat perforant path

Authors: S. E. HAMILTON, N. A. UPRIGHT, M. K. MOSES-HAMPTON, *J. J. RAMIREZ; Psychology Dept and Neurosci. Program, Davidson Col., Davidson, NC

Abstract: Long-term potentiation (LTP) has been investigated as a potential neurobiological mechanism of learning and memory. The hippocampal formation, particularly the dentate gyrus (DG), has been frequently utilized as a model structure for the exploration of memory. The perforant path (PP) arises in the entorhinal cortex and terminates in the ipsilateral dentate gyrus (DG). The septal input to the DG, termed the septodentate pathway (SD), projects from the medial septum/nucleus of the diagonal band of Broca and has important implications in hippocampal theta rhythm. The extent of the septum's role in hippocampal function has not been fully determined, particularly in regards to LTP in the PP response. Using an extracellular electrophysiological approach, we explored whether preliminary stimulation of the SD alters normal and LTP responses in the DG induced by PP stimulation. In male, Sprague-Dawley rats, stimulating electrodes were placed in the medial septum and medial EC unilaterally. Population spikes (popspikes) were recorded in the ipsilateral DG. An input-output curve of the PP response was determined prior to tetany, and recordings were made at the following intensities: 25%, 50%, 90%, and 100%. A heterosynaptic paired-pulse electrophysiological paradigm was performed to determine whether stimulation of the SD prior to PP stimulation could influence the PP response before and after tetany. The paired-pulse paradigm involved stimulation of the SD pathway (conditioning pulse) 60 ms prior to stimulation of the PP (test pulse). Paired-pulse recordings were made pre- and post-application of tetany (i.e. high frequency stimulation). Recordings continued for up to 120 minutes post-tetany. LTP was defined as an increase in popspike amplitude of at least 30% over the pre-tetany response. There was a significant interaction between the effects of stimulation intensity, tetany, and pairing on popspike amplitude in both the successful LTP and unsuccessful LTP groups. At 50% intensity in the successful LTP group there was a significant increase in popspike amplitude following tetany; however, in the unsuccessful LTP group at 50% there was a significant decrease following tetany. The cause of the depression in the unsuccessful LTP group is unclear. In both the successful and unsuccessful LTP groups examined at the 50% intensity, there were statistically significant increases in popspike amplitude due to heterosynaptic paired-pulse stimulation of the SD and PP both pre- and post-tetany. Therefore, the SD has the ability to facilitate the response of the normal and potentiated PP.

Disclosures: S.E. Hamilton: None. **N.A. Upright:** None. **M.K. Moses-Hampton:** None. **J.J. Ramirez:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R15NS078645

BYU Mentoring Environment Grant

BYU ORCA Grant

BYU PDBio Dept. Funding

Title: Running exercise mitigates the negative consequences of stress on hippocampal LTP

Authors: *R. M. MILLER, D. MARRIOT, J. TROTTER, R. DE ROQUE, D. LYMAN, T. HAMMOND, J. WELCH, A. FIELD, B. WALKER, N. CHRISTENSEN, D. HAYNIE, M. LEWIS, Z. BADURA, J. G. EDWARDS;
Physiol. and Developmental Biol., Brigham Young Univ., Provo, UT

Abstract: Cognition and memory in the mammalian brain can be impacted by behavior. For example, exercise and stress have positive and negative impacts respectively. While stress is anxiogenic and detrimental to neural function such as memory, exercise in contrast is anxiolytic and improves neural function. In the hippocampus, learning and memory are mediated at the cellular level by synaptic plasticity, known as long-term potentiation (LTP). It is now well established that stress decreases LTP and performance on behavioral memory assays while exercise enhances LTP and memory performance. What is not known however is whether exercise in association with stress can mitigate the negative impact stress has on memory. Therefore, we examined the effect exercise had on stress in the hippocampus using physiological, molecular, and behavioral techniques on C57BL/6 male mice. We conducted experiments on four groups: exercise without stress, sedentary without stress (control), exercise with stress, and sedentary with stress. Field electrophysiology confirmed that stress alone significantly ($P < 0.05$) reduced CA1 hippocampal LTP compared to sedentary controls and that exercise alone significantly increased LTP compared to controls. Importantly, we noted that mice that were exercised prior to stress exhibited LTP that was significantly greater than LTP for mice undergoing stress alone, but were not significantly different from control sedentary mice. Next, we used quantitative PCR to determine the differences in hippocampal mRNA expression of certain proteins that are part of the exercise and stress neural pathways. Specific proteins that were analyzed between the four groups were those in the brain-derived neurotrophic factor (BDNF) pathway, which others have demonstrated are upregulated in exercised mice, and glucocorticoid and mineralocorticoid receptors, which are upregulated in stressed mice. The CA1 region of the hippocampus is associated with spatial memory, so we used the radial arm maze to detect differences in spatial memory between the groups. Differences in time to complete a trial,

total distance traveled, and reference and working memory errors between the groups of mice were examined. Collectively, these results are significant as they suggest exercise as a suitable treatment to counteract the negative effects stress has on the hippocampus, specifically memory. By better understanding the neural pathways that are involved during exercise and stress, we can better understand how to use exercise to prevent everyday stresses from having a detrimental effect on cognition.

Disclosures: **R.M. Miller:** None. **D. Marriot:** None. **J. Trotter:** None. **R. De Roque:** None. **D. Lyman:** None. **T. Hammond:** None. **J. Welch:** None. **A. Field:** None. **B. Walker:** None. **N. Christensen:** None. **D. Haynie:** None. **M. Lewis:** None. **Z. Badura:** None. **J.G. Edwards:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.19/C26

Topic: B.08. Synaptic Plasticity

Title: Exercise training prevents hypobaric hypoxia induced neurodegeneration and synaptic strength

Authors: ***V. JAIN**[1,3], S. B. SINGH[2], K. RAVI[3];
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Abstract: Hypobaric hypoxia (HH) is an psychophysiological stress transpires at high altitude condition. Animal studies showed that chronic exposure to HH leads to neurodegeneration in hippocampus and other brain regions. Exercise training on other hand is known to enhance hippocampus associated neuronal function. In present study, we investigated the effects of treadmill running (exercise) on the memory task, neuronal morphology and level of synaptic proteins. 3 months old Sprague dawley rats were exposed to simulated HH condition in an Animal Decompression Chamber at an altitude of 25000 feet for 7 days. Four weeks of treadmill exercise training was given to rats before HH exposure as an intervention. Results showed that 4 weeks of treadmill running significantly increased the hippocampus learning memory and dendritic arbor of the CA1 neurons. Exercise Training also restored the level of synaptic proteins in hippocampus. Furthermore, exercise training reduced the neurodegeneration and prevents morphological alteration of neurons in CA1 region of hippocampus. In addition to above parameters neurogenesis was also studied in different groups and it was found that chronic HH

exposure significantly reduced hippocampal neurogenesis which further ameliorated in groups provided with exercise training before exposure. Taken together above findings, it can be concluded that providing exercise training can be proved beneficial against HH like stress conditions. Physical exercise may serve as a means to prevent deteriorating effect of stress which involves oxidative stress in their pathophysiology.

Disclosures: V. Jain: None. **S.B. Singh:** None. **K. Ravi:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.20/C27

Topic: B.08. Synaptic Plasticity

Support: NSF IOS-11212732

Title: Exercise reverses deficits in hippocampal long term potentiation in mice selected for high voluntary wheel running

Authors: *K. D. PARFITT[1], A. R. K. AYABE[2], K. GUAN[2], Z. THOMPSON[3], T. GARLAND, Jr.[3];
[1]Dept of Neurosci., Claremont, CA; [2]Neurosci., Pomona Col., Claremont, CA; [3]Biol., UC Riverside, Riverside, CA

Abstract: Motivation and exercise can influence synaptic plasticity, neurogenesis, BDNF release, and learning and memory, though the mechanisms have yet to be elucidated. In these studies, mice selected for high voluntary running (High Runner or HR mice) were used to examine effects of genotype and running on hippocampal long term potentiation (LTP), a cellular model for learning and memory. House mice (Mus domesticus) were bred for voluntary wheel-running for 73 generations; these mice now typically run 18 km/day, or twice as far as standard house mice and up to 2.7 times farther than control (C) mice. Slices prepared from HR mice that did not have access to running wheels showed significantly reduced LTP in area CA1 in response to a single theta burst stimulus, as compared to C mice, who showed robust potentiation in response to this mild stimulus (137.6 ± 7.6% vs 166.3 ± 7.2% of baseline, respectively). These LTP deficits in the HR mice were rescued, however, when the mice had access to exercise wheels (the magnitude of LTP was 221.3 ± 13.2% of baseline). The magnitude of LTP in slices from C mice with running wheels (single theta burst), and non-exercising HR and C mice in response to a stronger theta burst protocol (three theta bursts) was not significantly

different. Thus, it appears that the threshold for inducing LTP in the HR mice is altered, but the same levels of LTP can be achieved with sufficient stimulation. Input-output curves for HR and C mice, with or without access to running wheels, were not significantly different, indicating that neither the selection for voluntary wheel running nor running itself influences basal synaptic transmission. Similarly, there were no significant differences in paired-pulse facilitation between HR and C mice, suggesting that presynaptic differences between the mouse strains are unlikely. The LTP deficits observed in the HR mice, together with their hyperactive behavior, suggest that these mice may serve as a suitable animal model of attention deficit hyperactivity disorder (ADHD). Our observation that the LTP deficits could be reversed with exercise, and previous work showing that the intensity of running in HR mice can be reduced with ADHD medications such as methylphenidate, are consistent with this idea.

Disclosures: K.D. Parfitt: None. **A.R.K. Ayabe:** None. **K. Guan:** None. **Z. Thompson:** None. **T. Garland:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.21/C28

Topic: B.08. Synaptic Plasticity

Support: NMRC

Title: Neuroepigenetic blockade of HDAC3i reverses Alzheimer's pathology in murine hippocampus

Authors: *K. MUTHUKUMARAPPAN[1], T. BEHNISH[3], S. SAJIKUMAR[2];
[2]Physiol., [1]Natl. Univ. of Singapore, Singapore, Singapore; [3]Inst. of Brain Sci., Shanghai, China

Abstract: Histone deacetylase 3 (HDAC3) is known to play a critical negative role in learning and memory including in various pathological conditions. Considerable evidences support that sporadic AD and its progression are associated with epigenetic components leading to memory loss, cognitive decline, dementia and death. In this study, the attenuation of LTP by amyloid-β oligomer has been observed in the acute rat hippocampal slices which underlie the early synaptic dysfunction in AD. Modulation of chromatin structure through histone acetylation by HDAC inhibitors is reported for enhancing memory and synaptic plasticity, including its cellular correlate LTP. Thus, in this study, we retrieved amyloid-β oligomer induced LTP deficit by a

selective HDAC3i called RGFP 966, indicating a potential selective therapeutic candidate to treat cognitive decline in AD.

Disclosures: K. Muthukumarappan: None. **T. Behnish:** None. **S. Sajikumar:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Program#/Poster#: 671.22/C29

Topic: B.08. Synaptic Plasticity

Support: MH081935

National Academy of Sciences, Ford Foundation Postdoctoral Fellowship

Brain & Behavior Research Foundation NARSAD Young Investigator Award

Title: Heterosynaptic LTP of NMDA receptor-mediated transmission in the dentate gyrus

Authors: *A. RODENAS-RUANO, P. E. CASTILLO;
Neurosci., Albert Einstein Col. Med., Bronx, NY

Abstract: In the hippocampus, the dentate gyrus is a key relay station that controls the transfer of information from the entorhinal cortex to the hippocampus proper (CA1-CA4). This process requires that dentate granule cells (DGC) receive and integrate synaptic inputs from entorhinal cortex via perforant path, and proximal associational inputs from hilar mossy cells (MCs). Therefore, dentate gyrus function relies heavily on the integrative properties of DGC dendrites. N-methyl-D-aspartate receptors (NMDARs) can contribute to the integrative properties of neurons, and growing evidence indicates that NMDARs are dynamically regulated and subject to activity dependent long term plasticity. While early studies have shown that excitatory inputs onto DGCs can undergo LTP and LTD of NMDAR-mediated transmission, no study has addressed the input-specificity of this plasticity along dendritic axis. Here, we used an electrophysiological approach via whole-cell and field recordings in acute rat hippocampal slices, to investigate NMDAR plasticity rules at the three main excitatory inputs along the dendritic axis of DGCs [Lateral Perforant path (LPP)-DGC, Medial Perforant path (MPP)-DGC, and MC-DGC]. Using both high frequency stimulation and burst-timing dependent induction protocols, we found that only MPP-DGC, but not LPP-DGC nor MC-DGC, express NMDAR-LTP. We tested the possibility that NMDAR plasticity at MPP synapses could influence synaptic strength distally (LPP) and proximally (MC) along the dendrite, and found that induction of

NMDAR-LTP at MPP-DGC synapses heterosynaptically potentiates distal LPP-DGC NMDAR plasticity, but not MC-DGC synapses. We are currently investigating the molecular basis underlying input-specificity and heterosynaptic plasticity at DGC synapses. Our findings highlight a potentially key mechanism by which the dentate gyrus processes information that may contribute to hippocampal-dependent memory formation.

Disclosures: A. Rodenas-Ruano: None. **P.E. Castillo:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

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Topic: B.08. Synaptic Plasticity

Support: CIHR grant MOP 12046

Title: Characterization of *Aplysia* KIBRA, a conserved PKM stabilizing protein

Authors: *L. FERGUSON[1], S. CHEN[2], J. PARK[2], D. GLANZMAN[2], W. SOSSIN[1];
[1]Neurol. and Neurosurg., McGill Univ., Montreal, QC, Canada; [2]Dept. of Integrative Biol. and Physiol., Univ. of California, Los Angeles, CA

Abstract: The persistent activity of PKMs has been proposed to be critical to the maintenance of transcription-dependent memory, despite the fact that no links have been established between transcription and PKM maintenance. We have been studying the role of PKM in maintaining memories in the sensory-motor reflex of the marine mollusk, *Aplysia* californica. In this system, PKM formation is mediated through transcription-independent calpain-mediated cleavage of PKC into PKM. Indeed, PKMs are important in the maintenance of several forms of transcription-independent forms of intermediate facilitation (Sutton et al, 2004; Bougie et al, 2012). Nevertheless, similar to vertebrates, inhibitors of PKM can erase transcription-dependent forms of memory and synaptic facilitation, suggesting that transcriptional events can prolong the lifetime of PKM activity (Cai et al, 2011). We are examining the kidney-brain adaptor protein, KIBRA, as the link between transcription and PKM. KIBRA has been implicated in human episodic memory and has recently been shown to stabilize PKMs in rodent hippocampus and to be required for long-term memory formation (Vogt-Eisele et al., 2014). We have cloned the *Aplysia* orthologue of KIBRA and found that the PKM-binding domain (as well as the WW and C2 domains) is highly conserved between mammalian and *Aplysia* KIBRA. Quantitative PCR experiments demonstrate that KIBRA mRNA is upregulated during learning. Using an antibody

specific to *Aplysia* KIBRA, we will assess the upregulation of the KIBRA protein following 5HT-induced facilitation of *Aplysia* sensory/motor neurons. Finally, we will present results on whether KIBRA can stabilize PKMs formed during intermediate facilitation and if this depends on the PKM binding domain of KIBRA. Confirming KIBRA's role as the stabilizer of PKM responsible for prolonging the kinase's half-life in the potentiated synapse will broaden our understanding of the relationship between transcription-independent PKM formation and the corresponding transcription-dependent synaptic facilitation the kinase is responsible for maintaining.

Disclosures: L. Ferguson: None. **S. Chen:** None. **J. Park:** None. **D. Glanzman:** None. **W. Sossin:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

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Program#/Poster#: 671.24/C31

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R15NS078645

BYU Mentoring Environment Grants

BYU ORCA Grant

Title: Hippocampal stratum oriens interneurons express endocannabinoid biosynthetic enzymes and undergo anandamide-dependent potentiation

Authors: *L. N. FRIEND[1], R. WILLIAMSON[2], C. MERRILL[3], S. NEWTON[2], M. CHRISTENSEN[2], J. EDWARDS[2];
[1]Brigham Young Univ., Provo, UT; [2]BYU, Provo, UT; [3]UC Irvine, Irvine, CA

Abstract: The hippocampus is thought to mediate learning and memory by altering synaptic strength within its circuitry. In many cases, synaptic plasticity can be induced by signaling molecules such as lipid-based endocannabinoids like anandamide. Endocannabinoids modulate synaptic plasticity among hippocampal pyramidal cells and stratum radiatum interneurons; however, the role of endocannabinoids in mediating synaptic plasticity among interneurons in the stratum oriens is still unclear. These interneurons are feedforward inhibitory cells that have unique synaptic plasticity compared to those in the radiatum as they exhibit presynaptic LTP, rather than LTD. This plasticity is TRPV1 and nNOS independent. We therefore examined

whether oriens interneurons can produce endocannabinoids and whether these might be involved in presynaptic LTP. Using patch-clamp electrodes to extract single cells, we analyzed the expression of endocannabinoid biosynthetic enzyme mRNA using real-time, reverse transcription PCR. The cellular expression of several calcium-binding proteins and neuropeptides were used to determine interneuron subtype. We also analyzed cellular expression of several endocannabinoid biosynthetic enzymes, including N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), diacylglycerol lipase alpha (DAGLα), and 12-lipoxygenase, as well as type 1 metabotropic glutamate receptors (mGluRs). Preliminary data suggests that stratum oriens interneurons express mRNA for both biosynthetic enzymes and the type I mGluRs necessary for their production. We identified interneurons that coexpress mRNA for somatostatin and DAGLα as well as parvalbumin positive basket cells coexpressing NAPE-PLD, suggesting that both basket cells and O-LM cells, or another somatostatin-positive interneuron subtype, possess the enzymes necessary to produce various endocannabinoids. To test the role of endocannabinoids in synaptic plasticity, we performed whole-cell experiments and measured glutamate currents in the presence of a fatty acid amide hydrolase inhibitor (URB597; 1μM), which increases endogenous anandamide. Interestingly, URB597 potentiated stratum oriens interneurons in a CB1-dependent manner as it was blocked by AM-251 (2 μM). Collectively, this suggests oriens interneurons express the cellular machinery needed for endocannabinoid production, and can alter their plasticity in response to anandamide signal.

Disclosures: **L.N. Friend:** None. **R. Williamson:** None. **C. Merrill:** None. **S. Newton:** None. **M. Christensen:** None. **J. Edwards:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.25/C32

Topic: B.08. Synaptic Plasticity

Support: R01 DA17392

Title: Excitatory and inhibitory plasticity at an associative hippocampal circuit

Authors: ***K. R. JENSEN**, P. E. CASTILLO;
Albert Einstein Col. of Med., Bronx, NY

Abstract: The hippocampus contains two associative excitatory circuits; an auto-associative circuit formed by interconnected CA3 pyramidal neurons, and a hetero-associative circuit where

dentate granule cells (DGCs) synapse with glutamatergic hilar mossy cells (MCs) that project back to DGCs. While activity-dependent plasticity at CA3-CA3 synapses is well characterized, much less is known about synaptic plasticity in the hetero-associative circuit, in particular at the MC-DGC synapse. Given that a single MC contacts over 30,000 DGCs, plasticity at MC-DGC synapses is expected to have a profound effect on dentate gyrus output. MCs project their axons to a restricted region in the inner third of the molecular layer (IML). Interestingly, type 1 cannabinoid receptors (CB1Rs) are highly expressed on the presynaptic terminals of both MCs and hilar interneurons (INs) projecting to the IML, suggesting that endogenous cannabinoids (eCB) fine-tune proximal excitatory and inhibitory synapses. To test this possibility, and to examine activity-dependent MC-DGC plasticity, we used electrophysiology and optogenetics in acute rat hippocampal slices. While early *in-vivo* studies reported Hebbian, NMDA receptor-dependent LTP at MC-DGC synapses, we found that a pairing protocol (200 stimuli in IML, 2 Hz, Vh = 0 mV) elicited robust long term potentiation (LTP) in the presence of the NMDAR antagonist D-APV (50 μM). LTP was also elicited by selective activation of MC axons using optogenetics, making it unlikely that neuromodulatory fibers or CA3 back-projections contribute significantly to plasticity. MC-DGC LTP was associated with a significant decrease in paired-pulse ratio (PPR) and coefficient of variation (CV), strongly suggesting a presynaptic mechanism of expression. Previous work from our laboratory showed that MC-DGC synapses express endocannabinoid (eCB)-mediated short-term but not long-term depression (LTD) (Chiu & Castillo, 2008). To determine whether neighboring inhibitory inputs onto proximal DGC dendrites undergo eCB-mediated LTD (eCB-iLTD), we performed the following three manipulations: (a) theta-burst firing of DGCs; (b) transient application of the mGluR1/5 agonist DHPG (50 μM for 10 min); and (c) transient application of the CB1R agonist WIN55,212-2 (5 μM for 10 min). All three manipulations induced robust iLTD. Thus, by inducing LTD at inhibitory but not excitatory inputs, eCBs can shift the excitatory/inhibitory balance and regulate the DGC output. Activity-dependent plasticity at MC-DGC and neighboring IN-DGC synapses may play an important role in dentate gyrus-dependent memory formation.

Disclosures: K.R. Jensen: None. **P.E. Castillo:** None.

Poster

671. Long-Term Potentiation Signaling Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 671.26/C33

Topic: B.08. Synaptic Plasticity

Title: Kainate receptor activation regulates KCC2 function

Authors: *D. GARAND, M. WOODIN;
Cell and Systems Biol., Univ. of Toronto, Toronto, ON, Canada

Abstract: The potassium-chloride cotransporter 2 (KCC2) plays a critical role in inhibitory neurotransmission through its ability to maintain a low intracellular chloride level in the neuron. Surprisingly, KCC2 has also recently been found to interact with proteins involved in excitatory neurotransmission, including the kainate receptor (KAR) subunit GluK2. It is known that independent of kainate receptor activity, the physical interaction between KCC2 and GluK2 is important for KCC2 surface expression and oligomerization. However, it is unknown whether the activity of kainate receptors can directly influence KCC2 function. In this study we hypothesized that the activation of GluK2-containing kainate receptors would increase KCC2's ability to extrude chloride from the cell. We tested this hypothesis by performing slice electrophysiology experiments in the CA3 region of the hippocampus, recording E_{GABA} as a measure of KCC2 function. Activation of KARs with 1uM kainate, which activates both the canonical (ionotropic) and noncanonical (metabotropic) signalling pathways, produced a depolarization of the resting membrane potential, however this was accompanied by a significant hyperpolarization in E_{GABA}. This depolarization of resting membrane potential and hyperpolarization of E_{GABA} together produce a dramatic increase in the driving force for Cl- through the GABAA receptor, and thus an increase in GABAergic current amplitudes. Activation of KARs with 0.1uM kainate, which has been previously shown to selectively activate metabotropic signalling of KARs, produced an even larger hyperpolarization of E_{GABA}. This suggests that the two KAR signalling pathways exert different effects on KCC2. Metabotropic KAR signalling has been previously shown to decrease GABA release from interneurons, and consistent with this we found a decrease in conductance despite the hyperpolarization of E_{GABA}. Our findings demonstrate that activation of the kainate receptor is able to regulate KCC2 function, revealing a novel mechanism for excitatory: inhibitory balance.

Disclosures: D. Garand: None. **M. Woodin:** None.

Poster

672. Modulation of Neuronal Firing Properties II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 672.01/C34

Topic: B.10. Intrinsic Membrane Properties

Support: Prostate Cancer Canada

Michael G. DeGroote Institute for Pain Research and Care

Title: Ectopic activity of nociceptive sensory neurons in an animal model of cancer-induced pain

Authors: ***Y. ZHU**[1], E. SEIDLITZ[2], R. UNGARD[2], N. ZACAL[2], G. SINGH[2];
[2]Pathology & Mol. Med., [1]McMaster Univ., Hamilton, ON, Canada

Abstract: Background: Primary and metastatic cancers that affect bone are frequently associated with severe and intractable pain. The mechanisms underlying the development of cancer-induced pain remain unclear. The aim of this study was to determine whether ectopic activity of primary nociceptive sensory neurons contributed to peripheral sensitization and tumor-induced tactile hypersensitivity in cancer-induced pain. Methods: *In vivo* intracellular recording, immunofluorescent staining, and behavioral testing were used to investigate whether the intrinsic membrane properties and excitability of functionally-defined nociceptive dorsal root ganglion (DRG) neurons were altered in a rat model of bone cancer pain. Copenhagen rats were injected with 106 MATLyLu rat prostate cancer cells into the hind leg distal femur epiphysis to generate a bone cancer pain model. Once behavioural changes were detected using a von Frey tactile assessment, the animals were prepared for acute electrophysiological recordings of mechanically sensitive neurons in the DRG. Results: Our study showed that tumors growing in the femur produced significant tactile hypersensitivity in the ipsilateral hind paw. Furthermore, enhanced excitability of nociceptive (substance P expressing) DRG neurons was observed as a decreased threshold to activation of the peripheral receptive field, an excitatory discharge response to intracellular injection of depolarizing current into the soma, and an excitatory discharge response to electrical stimulation of the dorsal roots. Several alterations in intrinsic membrane properties were also observed in nociceptive DRG neurons, including: 1) depolarized resting membrane potential; 2) a dramatic decrease in amplitude and duration of evoked action potential; and 3) a dramatic decrease in amplitude and duration of the afterhyperpolarization. Conclusions: Our data suggest that injection of tumor cells into the distal epiphysis in rats induces an enhanced excitability of substance P-expressing DRG neurons. This enhanced excitability may be the result of alterations in intrinsic electrogenic properties of these neurons. Therefore, alterations in intrinsic membrane properties associated with the hyperexcitability of nociceptive sensory neurons appears to contribute to the peripheral sensitization and tumor-induced tactile hypersensitivity in cancer-induced pain.

Disclosures: **Y. Zhu:** None. **E. Seidlitz:** None. **R. Ungard:** None. **N. Zacal:** None. **G. Singh:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Program#/Poster#: 672.02/C35

Topic: B.10. Intrinsic Membrane Properties

Support: NIH NIAAA

VA Merit Review

Title: Alterations in neuronal function of central nucleus of amygdala in BK beta-1 or 4 knockout mice following chronic intermittent ethanol exposure

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Abstract: Large conductance Ca++ activated potassium (BK) channels associate with specific auxiliary subunits(encoded by four genes,β1-4) and BK channels associated with β1 and β1 subunits are expressed in central neurons. These BK channels regulate neuronal excitability and synaptic transmission. Neurons in the central nucleus of the amygdala (CeA) are involved in alcohol seeking and addiction, and BK channels are thought to be potential targets for ethanol action as presynaptic BK channels in the CeA mediate alcohol-induced GABA release. The accessory β1 and β4 subunits of BK channels are essential for modulating BK channel function and sensitivity to actions of ethanol; thus alterations in these subunits may influence the function of CeA neurons after both acute and chronic intermittent ethanol (CIE) exposure. Using genetic tools and whole-cell recordings in CeA neurons in an acute slice preparation, we have examined the membrane properties and inhibitory synaptic neurotransmission in CeA neurons from BK β1 or β4 KO and WT male mice following CIE exposure via a vapor inhalation chamber. Under whole-cell current clamp conditions, three distinct types of firing patterns (regular spiking, late spiking and low threshold firing) were recorded in neurons from these mice. No statistical differences in the resting membrane potentials and time constants between CeA neurons from WT and KO mice after CIE were observed. In CIE-mice, the mean input resistance of neurons from β4 KO mice was significantly higher than in neurons from WT mice. The threshold of action potentials determined by ramp current injection was not altered in CeA neurons lacking either β1or β4 in CIE-mice. In response to depolarizing current injections, β1 KO CeA neurons fire more spikes than WT neurons in CIE-mice but β4 KO CeA neurons appear to fire fewer spikes than WT neurons after CIE. Upon a strong depolarization, a depolarization block was also observed in some β4 KO CeA neurons from CIE-mice. Under whole-cell voltage clamp recording, both eIPSCs and sIPSCs were recorded in CeA neurons from β1 KO and WT CIE-mice. There was a significant increase in input/output curves of eIPSCs evoked by electrical stimulation in β1 KO CeA neurons of CIE-mice compared to WT CIE-mice. However, no significant differences in paired pulse ratio of eIPSCs (50 msec interval) or the mean frequency of sIPSCs of CeA neurons between β1 KO or WT CIE-mice were detected. Our current results

suggest that different BK channel accessory subunits in CeA neurons could regulate neuronal function following long-term intermittent exposure to ethanol, thus providing novel targets for pharmacotherapies aimed at reducing excessive ethanol consumption.

Disclosures: Q. Li: None. **C. Contet:** None. **S. Treistman:** None. **S.D. Moore:** None.

Poster

672. Modulation of Neuronal Firing Properties II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 672.03/C36

Topic: B.10. Intrinsic Membrane Properties

Support: DFG Grant JO 1079 1/1

Title: Characterization of spines undergoing ryanodine receptor induced enhancement of activity related calcium transients

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Abstract: Spines are dendritic protrusions that receive excitatory synaptic input from other neurons. Spines can also be depolarized by action potentials travelling back through the dendritic tree. The backpropagating action potential elicits calcium transients in dendrites and spines that are mostly mediated by voltage-sensitve calcium channels. Previously, we demonstrated that action potential firing enhances these backpropagating action potential evoked spine calcium transients, serving as a calcium memory of neuronal activity. The enhancement appeared compartmentalized to individual spines and was independent from enhancement of neighboring spines. We were able to define a calcium nanodomain created by intraspine ryanodine receptor activation mediated release of calcium from the intracellular stores. We aimed to further specify the spines that undergo the activity dependent enhancement with respect to development and dendritic localization. We performed two-photon calcium imaging on spines on proximal and distal dendritic segments of medial entorhinal cortex layer II cells. Spines located proximal as well as distally located spines did show an enhancement of the backpropagating action potential mediated calcium transient. Enhancement of spine calcium seems to be a spine specific process that is not dependent on the spines location in the dendritic tree. Rather intrinsic spine properties

determine the spine's potential for enhanced calcium signaling evoked by neuronal firing. All the results obtained so far were derived from measurements of animals in an age window were spine maturation is still ongoing and the number of spines is increasing. We therefore extended our measurements to older, more mature animals, where spines are generally considered less plastic. Significant enhancement was also observed in a more mature spine population.

Disclosures: **A. Theis:** None. **D. Schmitz:** None. **F.W. Johenning:** None.

Poster

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Topic: B.10. Intrinsic Membrane Properties

Support: CIHR

Dr. T.Chen Fong doctoral studentship

Title: HCN channels dysregulation leads to increased excitability of hippocampal neurons from PrP−/− mice

Authors: ***J. FAN**, P. STEMKOWSKI, S. A. G. BLACK, I. A. SOUZA, G. W. ZAMPONI; Physiol. and Pharmacol., Univ. of Calgary, Calgary, AB, Canada

Abstract: It is well established that the loss of cellular prion protein (PrP) function is involved in a variety of electrophysiological abnormalities that contribute to epileptogenesis. We have previously shown that synaptic activity in hippocampi of PrP-null mice is increased due to enhanced N-methyl-D-aspartate (NMDA) receptor function. We now examined the intrinsic firing pattern of cultured hippocampal neurons. Using whole-cell voltage-clamp and current-clamp recordings from mature primary hippocampal cultures, the effect of PrP deletion on intrinsic neuronal excitability and ion channel activity was examined. We observed that the absence of PrP profoundly affected the firing properties of hippocampal neurons in the presence of synaptic blockers, increasing the number of action potentials (APs) ($p < 0.05$), decreasing the spike threshold ($p < 0.001$) and reducing cumulative AP latencies ($p < 0.05$). Interestingly, only membrane impedance, among tested active and passive parameters, was observed to be different, which was greater in PrP-null neurons ($p < 0.05$). To determine whether Ih may serve as an underlying ionic mechanism, HCN channel activity was examined. In current clamp recordings, the amplitude of voltage sag in PrP-null neurons decreased in response to hyperpolarizing

current injection ($p < 0.05$). In voltage clamp, a series of hyperpolarizing test potentials revealed that PrP-null neurons exhibited a decrease in Ih peak current ($p < 0.05$), along with a hyperpolarizing shift in half activation voltage. In addition, we observed that the time course of Ih activation became significantly slower in PrP-null neurons over a command voltage range ($p < 0.05$). To determine whether the difference in membrane impedance resulted from a decrease in Ih activity, we assessed changes in membrane impedance following bath application of ZD7288. The membrane impedance in WT and PrP-null neurons became indistinguishable in the presence of ZD7288. Despite the upregulation of Ih, HCN1 and HCN2 protein levels remained similar and neither HCN1 nor HCN2 was found to form a complex with PrP. These data suggest that the absence of PrP causes a downregulation in the activity of HCN channels, which in turn increases membrane impedance to potentiate intrinsic excitability of hippocampal pyramidal cells.

Disclosures: J. Fan: None. **P. Stemkowski:** None. **S.A.G. Black:** None. **I.A. Souza:** None. **G.W. Zamponi:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Program#/Poster#: 672.05/C38

Topic: B.10. Intrinsic Membrane Properties

Support: 239192 SLC, EJG

Title: Chronic toluene exposure alters the neural activity in medial prefrontal cortex of adolescents rats

Authors: *M. ARMENTA-RESÉNDIZ, S. L. CRUZ, E. J. GALVAN;
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Abstract: Toluene is an abused solvent used mainly among adolescents for its psychoactive effects. It has been documented that chronic exposure to toluene-based products can produce severe cognitive deficit. Although there are a few studies regarding the electrophysiological effects of acute toluene exposure, little is known on the possible adaptations that result from chronic exposure in specific brain areas. This study examined the effects of intermittent chronic toluene exposure on intrinsic excitability and spontaneous synaptic activity of pyramidal neurons in medial prefrontal cortex (mPFC) of adolescent rats. Individual male Wistar rats were exposed for 30 min in a static exposure chamber to air or 8000 ppm toluene twice a day for 10 days from postnatal day 22 to 35. After completing the exposure period, rats were decapitated and layer V

pyramidal neurons were recorded from medial prefrontal cortex slices using whole-cell patch-clamp recordings. Toluene did not change the resting membrane potential, but altered other measures of intrinsic excitability; in particular, it reduced neuronal input resistance, increased the I-V curve rectification phase and decreased the rheobase current to induce action potentials. Toluene also augmented neuronal firing frequency without altering the kinetic of action potential and increased the frequency, but not the amplitude, of spontaneous excitatory postsynaptic currents (EPSCs). Taken together, our results show that repeated exposure to a behaviorally relevant concentration of toluene increases the excitability of layer V pyramidal neurons in mPFC in adolescent rats.

Disclosures: **M. Armenta-Reséndiz:** None. **S.L. Cruz:** None. **E.J. Galvan:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Topic: B.10. Intrinsic Membrane Properties

Support: James S. McDonnell Foundation

Title: Clarithromycin increases neuronal excitability by reducing gaba-ergic signaling

Authors: ***E. K. BICHLER**[1,2], C. C. CRON[2], P. S. GARCÍA[1,2];
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Abstract: Antibiotics are used in the treatment and prevention of bacterial infections but side effects such as convulsions can limit their use. The adverse effects of cephalosporin on neuron excitability has been recognized for decades, however recently we demonstrated that administration of clarithromycin improves daytime sleepiness in hypersomnic patients known to have enhanced $GABA_A$ signaling (Trotti et al., 2014). Similarly, our laboratory has previously shown that clarithromycin decreased inhibitory chloride currents in HEK 293 cells expressing recombinant human $GABA_A$ receptors (Garcia and Jenkins, 2009). In order to explore the potential application of clarithromycin as a stimulant, we performed whole-cell patch-clamp recordings in rat pyramidal cells from the CA3 region in acute hippocampal slices. We hypothesized that by reducing $GABA_A$ receptor activation clarithromycin would increase neuron excitability. In the presence of 300uM clarithromycin, rheobase current was reduced to 50 % of controls (measured as the smallest required step current injected at the soma that results in an action potential), F-I relationship (number of action potentials as a function of injected current)

was shifted to the left and the resting membrane potential was significantly more depolarized compare to controls (-55 mV vs. -65mV; $p<0.001$). Additionally, we tested the effect of clarithromycin in an *ex vivo* seizure model (high K^+ and low Mg^+ solution) by evaluating its effect on spontaneous local field potentials in stratum pyramidale of the CA3 hippocampal region. Bath application of clarithromycin enhanced burst frequency 2-fold compared to controls ($p<0.05$). Taken together, these results suggest that blocking GABA-ergic signaling by clarithromycin increases cellular excitability and cause higher susceptibility to seizure. The stimulatory properties of clarithromycin may have a positive impact on clinical applications such as emerging from anesthesia and promoting vigilance in hypersomnic patients. However, the administration of clarithromycin should be carefully considered in patients with seizure disorders.

Disclosures: **E.K. Bichler:** None. **C.C. Cron:** None. **P.S. García:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Program#/Poster#: 672.07/C40

Topic: B.10. Intrinsic Membrane Properties

Support: BBSRC

Title: ATP depletion supresses action potential firing independently of synaptic transmission

Authors: ***S. J. LUCAS**[1], C. B. MICHEL[2], Y. SWEENEY[3], M. H. HENNIG[3], B. P. GRAHAM[2], I. D. FORSYTHE[1];
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Abstract: Information transmission requires high levels of energy to maintain ionic gradients, Ca^{2+} extrusion and vesicular recycling. There is a large metabolic cost for restoring Na^+ and K^+ gradients following action potential generation and conduction. Hence at times of high activity and in brain regions, such as the auditory pathway, with high metabolic rates, the balance between energy consumption and maintenance of neuronal function may compromise information transmission. Many studies of metabolism in brain slices have used established pharmacological protocols to inhibit ATP production, but do not differentiate between suppression of neuronal excitability and synaptic transmission. The calyx of Held/MNTB synapse in the auditory brainstem, which transmits information at high frequencies and would be

expected to require high levels of ATP, provides a model at which to study the effects of ATP depletion on neuronal excitability and transmitter release, independently and at the level of a single synaptic connection. Using whole-cell recordings from MNTB neurons, in auditory brainstem slices from P14-18 mice, we have studied the effects of ATP depletion protocols on neuronal function at the calyx of Held/MNTB synapse. Sodium azide, an inhibitor of mitochondrial function, together with 2-deoxy-D-glucose (2-DG), an inhibitor of glycolysis, are commonly used to induce a chemical hypoxia. As expected we found that depletion of ATP with perfusion of 5 mM sodium azide and 5 mM 2-DG for 8 min depolarises neurons and inhibits low frequency (0.1 Hz) synaptic transmission. This ATP depletion, however, also results in a loss of action potential firing; hence the loss of postsynaptic responses can be due to to a failure in presynaptic action potential conduction rather than a true synaptic failure of transmitter release. Removal of energy substrates from the perfusing aCSF similarly impairs firing, with some neurons appearing more vulnerable to metabolic impairment. Preceding the loss of firing, neurons show early changes in action potential threshold, which is followed by a decrease in action potential amplitude while the half-width increases. Using a mathematical model of the calyx of Held synapse coupled with metabolic pathways, the contributions of ATP depletion to impairments of neuronal function were predicted. The impairment in action potential firing following ATP depletion has important implications when attempting to study energy depletion on synaptic function: it confounds the interpretation of studies that investigate hypoxia or energy depletion using indirect measures of synaptic function, such as the network activity and field potential measurements.

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Poster

672. Modulation of Neuronal Firing Properties II

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Topic: B.10. Intrinsic Membrane Properties

Support: JSPS Grant Scientific Research (B) (25293409)

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Title: Neuropeptide Y modulates the spike discharge characteristics in mesencephalic trigeminal neurons

Authors: S. SEKI, *S. TANAKA, Y. ONO, T. TSUJI, M. KOGO;
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Abstract: Background and purpose: Neuropeptide Y (NPY) is one of neuropeptides with powerful orexigenic effect. Our recent study has demonstrated that intracerebroventricular administration of NPY induced increase of food intake in a dose-dependent manner, while feeding rate was decreased in a higher concentration (Ushimura et al., 2015). In addition to a role on the feeding behavior, NPY also has integral effects on neuronal systems related to other spontaneous behaviors such as hyperactivity, rearing, grooming. Previous studies have revealed that 1) the rat trigeminal complex, including the trigeminal motoneurons and mesencephalic trigeminal neurons (MTN), display rich immunoreactive staining for both the NPY-Y1 and -Y5 receptors (Wolak et al., 2003), 2) The intrinsic membrane properties of MTN are critical for producing rhythmical oral motor activities (Tanaka and Chandler, 2006; Tanaka et al., 2003). Therefore, in the present study, we examined the potential for NPY-induced modulation of membrane excitability in MTN underlying rhythmical jaw movements. Methods: Coronal brain-slices were prepared from Sprague-Dawley rats (P3-19) and immersed in oxygenated normal ACSF, then whole-cell configuration was obtained from MTN using patch-clamp technique. Patch-electrodes with 3-4㏁ resistance were filled with K-gluconic acid-containing normal solution. Voltage and current signals were digitized and recorded using pCLAMP acquisition software (v9.0, Molecular Devices). Liquid junction potential between bath and pipette solutions was not corrected off-line. Results and Conclusions: Bath application of NPY (0.1 μM) depolarized the membrane potential and induced inward current in MTN, which was persisted in the presence of TTX and dependent on external Na+ and Ca2+. The duration of AHP following an action potential was significantly shortened and the mean spike frequency in the repetitive firing activity was consistently increased by NPY. In addition, intrinsic bursting activities induced by maintained current injection showed decrease of burst duration and significant increase of bursting frequency after application of NPY, indicating the excitatory effects of NPY on spike discharge characteristics in MTN. Further experiments revealed an involvement of both Y1 and Y5 receptor activations in the modulatory effects of NPY.

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Poster

672. Modulation of Neuronal Firing Properties II

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Topic: B.10. Intrinsic Membrane Properties

Title: Spike amplification by the afterhyperpolarization; a possible mechanism of short term synaptic plasticity?

Authors: *N. KUCZEWSKI, N. FOURCAUD-TROCMÉ, S. GARCIA, P. DUCHAMP-VIRET;
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Abstract: The modification of the action potential (AP) waveform is well known as affecting the quantity of neurotransmitter released at the synaptic level. Since the beginning of the 21th century, accumulating evidences showed that AP waveform and the following synaptic transmission can be affected by modifications of the neuronal resting potential (Vrest). Experimentally these modifications are obtained either, by somatic current injection or, by some peculiar form of physiological activity, such as the cortical up and down states. However, in these studies, the AP waveform changes were induced by important (several dozen of mV) and relatively long lasting modification of somatic Vrest. Moreover, as a consequence of the spatial attenuation, the synaptic effect of the somatic Vrest modifications were limited to proximal synapses. Here, in rat olfactory bulb slices, we reported that, in Mitral Cells (MCs), the small hyperpolarization generated by the post spike afterhyperpolarization (AHP) increases both the AP amplitude and duration during spontaneous firing activity. Preliminary results suggest that this amplification results from the hyperpolarization-induced recovery from inactivation of sodium and T-type Ca2+ channels and, that such an effect is partially counterbalanced by the contemporary recovery from inactivation of potassium channels. As a consequence of the AHP short duration, the spike amplification is only observed when two successive APs are generated at short time intervals (<100 ms); thus, the occurrence of the amplification phenomenon increases with the MC instantaneous firing frequency. Preliminary data suggest that the AHP-induced AP amplification is capable to increase the release of glutamate from MC dendrites, suggesting that the small variations of somatic Vrest produced by the AHP are capable to affect AP waveform in the dendritic three. This could be the achieved without spatial attenuation thanks to the AP regenerative properties that it supposed to allow the AHP propagation throughout the neuronal processes. Ongoing experiments are testing this hypothesis. Our results open the possibility that the frequency-dependent modifications of AP waveform produced by the AHP could participate in the changes of the synaptic transmission responsible of short term synaptic plasticity.

Disclosures: N. Kuczewski: None. **N. Fourcaud-Trocmé:** None. **S. Garcia:** None. **P. Duchamp-Viret:** None.

Poster

672. Modulation of Neuronal Firing Properties II

Location: Hall A

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Program#/Poster#: 672.10/C43

Topic: B.10. Intrinsic Membrane Properties

Title: Strongly enhanced activin signaling after electroconvulsive seizures impacts on granule cell excitability

Authors: F. ZHENG[1], A. S. LINK[1], *C. ALZHEIMER[2];
[1]Physiol. and Pathophysiology, [2]Univ. Erlangen-Nuremberg, Erlangen, Germany

Abstract: Activin A is a member of the transforming growth factor-beta family. In the nervous system, it was originally identified as a neurotrophic and neuroprotective factor, but recent evidence from our and other laboratories showed that activin also tunes excitatory and inhibitory neurotransmission in the brain in a fashion that impacts on cognitive functions and affective behavior. In addition to the canonical pathway, which involves activin binding to heteromeric receptor complexes and subsequent phosphorylation of the intracellular effector proteins SMAD-2/3, activin receptors might also act on other signaling systems, in particular mitogen-activated protein kinase (MAPK) signaling. In neuropsychiatry, activin A has been implicated in the regulation of mood disorders and has been suggested to serve as an endogenous antidepressant. In fact, activin signaling is targeted by the two prevailing treatment strategies in major depression, antidepressant drugs and electroconvulsive therapy (ECT), but it is still unknown how activin might produce its putative therapeutic benefits. Here we report that electroconvulsive seizures (ECS, 25 mA for 0.5 s at 50 Hz), the mouse analog of ECT in humans, strongly induced activin signaling in the granule cell layer of the dentate gyrus of mouse hippocampus. Whole-cell and field potential recordings from brain slices, prepared 12 h post ECS, showed that granule cell excitability was greatly enhanced. Linking the electrophysiological effects of ECS on granule cell excitability to the accompanying rise in activin in the same hippocampal region, we found that incubation of control slices with activin (50-100 ng/ml) for 3-6 h closely mimicked the features of the ECS-mediated increase in granule cell excitability. The activin-induced enhancement of granule cell firing was abolished by the activin-binding protein follistatin 288, but not by SB 421532, which interferes with the canonical activin signaling by inhibiting SMAD phosphorylation. Furthermore, the effect of activin on granule cell firing was greatly truncated in the presence of the ERK-MAPK inhibitor PD 98058, indicating the involvement of the non-canonical pathway. Our results suggest that enhanced activin signaling might be responsible for a functionally significant effect of ECS in dentate granule cells and that the underlying mechanism might involve a SMAD-independent pathway.

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Poster

672. Modulation of Neuronal Firing Properties II

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Topic: B.10. Intrinsic Membrane Properties

Title: Measurement and simulation of temperature effects on axonal conduction, synaptic transmission and network activity - Implications for the evolution of hibernation

Authors: *T. BULLMANN[1], K. DELIGKARIS[1], A. HIERLEMANN[2], U. FREY[1];
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Abstract: Temperature plays a fundamental role in physiology, determining the rate of biochemical processes. This dependence is usually expressed by using the Q10 temperature coefficient. In the nervous system, temperature affects the ion channel kinetics, axonal conduction, calcium signaling, synaptic release, binding of transmitters to receptors, for which the Q10 values diverge and range between 2~3. This divergence poses a problem if the function of the nervous system as a whole must be maintained over a large temperature range, e.g., in cold-blooded animals. In the stomatogastric nervous system of crabs, the rhythmic motor pattern is well preserved within the physiological temperature range (7~23°C, ΔT=16°K; Tang et al., 2012). This robustness can be explained by 'sloppy' parameter sensitivity and similar Q10 values (Caplan et al., 2014). In most warm-blooded animals, with the exception of animals that hibernate, the physiological temperature range is small (typically 35~38°C, ΔT=3°K), and so should be the 'permissible' temperature range. We cultured primary neurons from rat on high-density microelectrode arrays (HD-MEA; 11016 electrodes). Surprisingly, network recordings revealed stereotypical bursts over a wide temperature range (15~40°, ΔT=25°K). Bursts retained their temporal structure despite diverging temperature dependence of inter spike intervals, number of spikes/bursts, burst periods and burst lengths. During cooling, bursts were observed to be increasingly stretched in time, disappeared below 15°C, but reappeared upon warming, and then showed again the same patterns, even after prolonged cooling down to 10°C. We simulated the temperature dependence of network bursting by introducing a Q10=3 into the activation of the slow component of a simple neuron model. In a next step, we want to include axonal conduction delays and spike-time-dependent plasticity, which leads to repeated spike patterns (polychronization; Izhikevich, 2006). Therefore, we recorded action potentials along full axonal arbors at subcellular resolution (3,150 electrodes/mm2) over a similarly temperature range (20~40°C). Together with imaging of cooling-induced synapse regression this will allow us to

build a richer model of the temperature dependence by incorporating Q10 values for several crucial parameters. From an evolutionary standpoint, the wide 'permissible' temperature range and the stability after prolonged cooling is surprising for warm-blooded animals. This low-temperature tolerance might represent a plesiomorphic trait, which has been previously hypothesized from the patchwork distribution pattern of hibernators in the phylogeny of mammals.

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Poster

672. Modulation of Neuronal Firing Properties II

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Topic: B.10. Intrinsic Membrane Properties

Support: Swartz Foundation

MH 46742

Title: Uncovering cellular properties from network dynamics

Authors: ***J. GJORGJIEVA**, G. DRION, E. MARDER;
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Abstract: Functionally equivalent circuits can generate similar activity patterns despite disparate intrinsic neuronal and synaptic properties. One way to alter these properties is through neuromodulatory substances, which can reconfigure circuits and produce multiple behavioral outputs. Here we use computational models to investigate the role of descending neuromodulatory inputs in regulating the output of a simple circuit: a half-center oscillator. We interpret our results in the context of the crustacean stomatogastric nervous system using conductance-based model neurons for spike generation with intrinsic and synaptic conductances chosen to reproduce the properties of gastric mill neurons. We focus on the action of modulatory commissural neuron 1, MCN1, which provides excitatory input to a pair of gastric mill neurons, LG and Int1. First we examine how the spatiotemporal input structure influences the rhythm generated by the half-oscillator. Because descending inputs from modulatory neurons on the two sides of the animal may not be identical, we examine the transfer of input correlations of varying strength for networks with a different composition of intrinsic and synaptic

conductances. In the case when similar network activity arises from disparate cellular intrinsic properties, we use these results to determine conditions to read out the different cellular properties from network output. For this purpose we compare model neurons whose biophysical composition endows them with very different firing patterns, for instance spiking versus intrinsically bursting. When coupled in half-center oscillator networks, cellular identity becomes hidden and the two networks generate similar patterns. Different network patterns emerge only when driving the networks with neuromodulatory inputs with an appropriate structure. This approach enables us to uncover single cell identity from network activity in a manner that can be applied to other circuits. It also allows the use of correlated descending input to interpret trade-offs between synaptic and intrinsic properties in governing circuit output.

Disclosures: J. Gjorgjieva: None. **G. Drion:** None. **E. Marder:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Research School Plus

Title: Contribution of different neuromodulators on persistent firing in hippocampal CA1 pyramidal cells

Authors: *M. J. VALERO-ARACAMA, M. M. SAUVAGE, M. YOSHIDA;
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Abstract: Persistent firing, a neuronal firing activity that outlasts triggering stimulation, has been observed in neurons from multiple brain regions *in vivo*. The function of persistent firing in the hippocampus is believed to be information maintenance, which is required for hippocampal dependent temporal association tasks. We have recently shown that principal neurons of the rat hippocampal CA1 area maintain persistent firing *in vitro*, supported by the calcium activated non-specific cationic (CAN) current, in the presence of the cholinergic receptor agonist carbachol. While cholinergic receptor dependency of persistent firing is in line with the importance of acetylcholine in temporal association tasks, other neuromodulators that also play a

crucial role in memory, such as noradrenaline and serotonin may contribute to persistent firing as well. In fact, an *in vitro* work has recently shown that noradrenaline facilitates persistent firing in the prefrontal cortex. However, the effects of these neuromodulators on persistent firing in CA1 pyramidal cells remain largely unknown. In this study, we report the effects of noradrenaline and serotonin on persistent firing either by applying them alone or in combination with carbachol using *in vitro* whole cell patch clamp recordings from mouse CA1 pyramidal cells.

Disclosures: **M.J. Valero-Aracama:** None. **M.M. Sauvage:** None. **M. Yoshida:** None.

Poster

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Title: Intrinsic properties of mouse entorhinal cortex layer ||/||| interneurons and principal cells identify seven functional groups

Authors: ***M. FERRANTE**[1], B. TAHVILDARI[3], A. DUQUE[3], D. SALKOFF[3], E. W. ZAGHA[3], M. E. HASSELMO[2], D. A. MCCORMICK[3];
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Abstract: Inhibitory interneurons are an important source of synaptic inputs (i.e., LII/III Stellate Cells and Pyramidal Cells) that may contribute to network mechanisms for coding of spatial location by grid cells. The intrinsic properties of inhibitory interneurons in the entorhinal cortex (EC) are mostly undescribed. We recorded a range of intrinsic properties (i.e., resting membrane potential, input resistance, and time constants; AP threshold, half-height-width, amplitude, latency, and frequency adaptation; F-I and V-I curves; voltage phase plots, SAG, and dv/dt ratios) from 5 classes of EC interneurons identified by molecular biomarkers (i.e., RCan2, SOM, 5HTR3a, VIP, and NPY) and from 2 classes of EC principal cells (i.e., stellate cells and pyramidal cells). We report a broad physiological diversity between and within different cell classes in the EC. To better understand the source of this intrinsic variability we applied supervised and unsupervised methods of hierarchical cluster analysis to functionally classify neurons. The analysis revealed 7 physiologically-derived cell types in the superficial layers of

the EC that mostly corresponded to the mouse lines identified by biomarkers. For instance, most of the stellate cells, pyramidal cells, and RCan2 cells formed distinct functional clusters that did not include other cells but themselves. Despite these similarities, the functional analysis revealed a few unexpected and important differences. For instance, SOM+ cells were divided in two functional groups, one of these sub-groups (that included 26 of the 34 SOM+ cells) was intrinsically more similar to NPY+-Non-NGF cells than to the other SOM+ sub-group. Similarly, while most of the 5HTR3a+ (27 out of 39) were functionally related to most VIP+ cells (16 out of 19), about 13% of 5HTR3a+ cells functioned like NPY+-NGF interneurons. Finally, we reduced the complex multi-dimensional space of intrinsic properties to the most salient five (i.e., spike frequency adaptation, SAG, dV/dt, membrane time constant, and AP threshold) that predicted with high accuracy (>90%) the cellular biomolecular identity based on their intrinsic properties. We also report immunohistochemical cell counts and neuronal morphological reconstructions. Our results provide a framework for classification of functional subtypes of cortical neurons by their intrinsic membrane properties.

Disclosures: **M. Ferrante:** None. **B. Tahvildari:** None. **A. Duque:** None. **D. Salkoff:** None. **E.W. Zagha:** None. **M.E. Hasselmo:** None. **D.A. McCormick:** None.

Poster

672. Modulation of Neuronal Firing Properties II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 672.15/C48

Topic: B.10. Intrinsic Membrane Properties

Support: NIH R01 MH085074

NIH R01 EB016407

Title: Input/output properties of interneurons in the medial entorhinal cortex

Authors: ***J. J. MARTINEZ**[1,2], J. A. WHITE[2];
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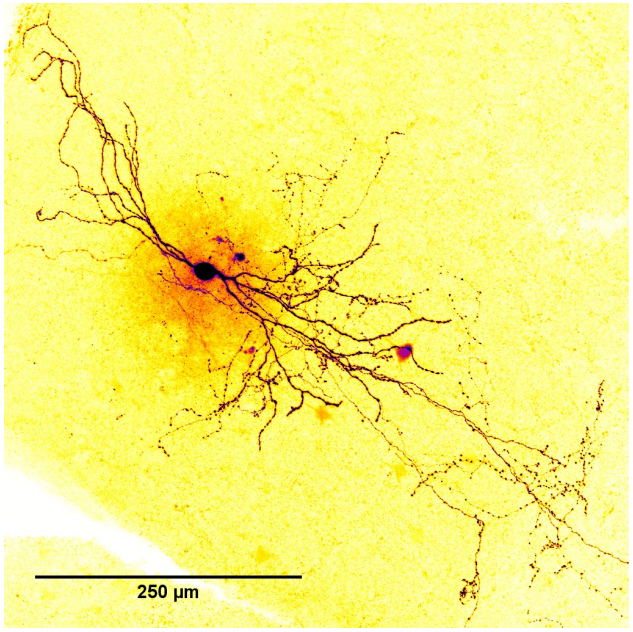
Abstract: Local GABAergic interneurons regulate the activity of spatially-modulated principal cells in the medial entorhinal cortex (MEC), mediating stellate-to-stellate connectivity and enabling grid formation via recurrent inhibitory circuitry. Despite the important role interneurons seem to play in the MEC cortical circuit, the combination of low cell counts and functional diversity has made systematic electrophysiological studies of these neurons difficult. There thus

remains a paucity of knowledge on the electrophysiological profiles of superficial MEC interneuron populations. Using GAD2-Cre and PV-Cre genetic markers to label GABAergic cells throughout the cortex, we targeted GABAergic interneurons for whole cell patch clamp recordings and characterized their passive membrane features, basic input/output properties and action potential shape. These electrophysiologically characterized cells were then anatomically reconstructed, with emphasis on axonal projections, soma morphology, and localization within the MEC DV axis. The pial depth of each neuron and the spatial extent of axonal projections were used to cluster interneuron populations. This method was used determine the electrophysiological profile of anatomically distinct interneuron groups in the MEC, including but not limited to basket/chandelier cells, multipolar, horizontal and bipolar cells. Basket cells in the MEC were shown to fire at the fastest rate relative to other interneurons, and did not show any significant difference along the dorsal ventral axis in any electrophysiological measurements. Superficial horizontal layers, in turn, fired at slower rates than most other interneuron populations. These results, among others, will provide greater understanding of the electrophysiological characteristics of MEC interneurons, help guide future *in vivo* studies, and aid in uncovering the mechanism of grid field formation.



Disclosures: **J.J. Martinez:** None. **J.A. White:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant MH071739

NIH Grant GM058234

Title: BK channel alternative splicing contributes to homeostatic adaptation of neuronal excitability

Authors: *B. LI, B. SUUTARI, R. W. TSIEN;
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Abstract: Homeostasis of intrinsic excitability is an important capability of CNS neurons to keep neuronal firing away from extremes of frequency and thus protect the information transfer capacity of brain circuits. Our previous study showed that chronic inactivity increased action potential width, but the underlying mechanism has not been elucidated. Here, we found that BK channel, an important regulator of action potential shape and neuronal excitability, undergoes alternative splicing (AS) of exon 29 (E29) in response to 48 hr TTX treatment of cultured cortical neurons: E29 levels dropped to ~50% of control without a significant change in total BK channel mRNA. In turn, neurons overexpressing BK channels lacking E29 showed wider action potentials than those expressing BK channels including E29, suggesting that TTX-induced E29 skipping might contribute to regulation of excitability. The AS of E29 was regulated by Nova-2, which directly binds to the intron downstream of E29. Indeed, overexpression of Nova-2 induced E29 inclusion in E29 minigene-transfected N2A cells. Moreover, knockdown of Nova-2 strongly reduced E29 inclusion in cortical neurons. These data suggest that Nova-2 is both sufficient and necessary for E29 inclusion. Chronic inactivity triggered Nova-2 nuclear export, apparently suppressing E29 inclusion. Mechanistically, we found that blockade of Ca_V1 channel by nimodipine, inhibition of CaMKs by KN-93, inhibition of CaMKK by STO-609 and buffering of nuclear CaM by CaMBP4(nu) blocked TTX-induced Nova-2 translocation and regulation of E29 splicing, indicating that long term inactivity-induced activation of a Ca_V1-CaMKIV pathway mediates this process. Indeed, 48 hr TTX treatment induced activation of CaMKII and CaMKIV, and overexpression of constitutively active CaMKIV recapitulated TTX-induced Nova-2 translocation. Furthermore, we found that CaMKIV directly binds to and phosphorylates Nova-2. Constructs mimicking the phosphorylation by CaMKIV induced Nova-2 translocation. It has been suggested that abnormal homeostatic adaptation contributes to autism. To this end, we demonstrate that neurons from Timothy Syndrome mice, a widely studied autism model induced by Cav1 channel point mutation, express a significant lower level of E29 (<50% of control) after chronic TTX treatment. This effect was reversed by nimodipine, KN-93 and STO-609, indicating that the atypical E29 splicing in TS neurons reflects hyperactivity of the Ca_V1-CaMKIV pathway in response to inactivity. These results suggest that abnormal homeostatic adaptation of E29 AS contributes to this highly penetrant form of autism.

Disclosures: **B. Li:** None. **B. Suutari:** None. **R.W. Tsien:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Program#/Poster#: 672.17/C50

Topic: B.10. Intrinsic Membrane Properties

Support: NIH P50 MH103204

Title: Pyramidal neuron heterogeneity in the monkey posterior parietal and dorsolateral prefrontal cortices

Authors: ***G. GONZALEZ-BURGOS**, T. MIYAMAE, D. ARION, D. A. LEWIS; Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The functional specialization of primate neocortical areas may depend on area-specific neurophysiological and morphological properties, partly determined at the transcriptional level. Layer 3 pyramidal neurons (L3PNs) play a crucial role in neocortical circuit function, by integrating inputs from multiple sources and generating output signals conveyed onto other cortical regions via their long-distance axonal projections. In sensory versus association areas of the primate neocortex, L3PNs have significantly different properties that could contribute to area-specific function. However, it is not known if L3PNs show different features between the dorsolateral prefrontal (DLPFC) and posterior parietal (PPC) areas of the primate neocortex, which are distinctively involved in cognition, but co-activate in various cognitive tasks and communicate via reciprocal connections. We therefore began comparing the electrophysiological, morphological and transcriptional properties of L3PNs from the macaque monkey DLPFC and PPC. In whole-cell recordings from L3PNs in acute slices from DLPFC or PPC, we assessed the intrinsic membrane properties via the response to injection of current steps. Recordings from L3PNs (n=20 DLPFC and n=19 PPC) showed that, in both areas, L3PNs were divided into regular spiking (rsL3PNs) or burst spiking (bsL3PNs) subtypes (most bsL3PNs had weak bursting properties). Two Way ANOVA (rsL3PNs/bsL3PNs x DLPFC/PPC) for 9 intrinsic electrophysiology parameters showed that rsL3PNs and bsL3PNs had similar properties, but differed in the amplitude of the single-spike afterhyperpolarization and the hyperpolarizing response sag. No significant effects of cortical area on intrinsic properties were found thus far, but, interestingly, the proportions of L3PN subtypes differed between DLPFC (rsL3PNs:bsL3PNs, 12:8) and PPC (rsL3PNs:bsL3PNs, 17:2; p=0.035, Chi-Square test), suggesting greater heterogeneity of input/output transformation properties in DLPFC. Ongoing

work is assessing the morphological properties of the L3PNs filled with biocytin during recording and the transcriptional profile using DNA microarray amplification in RNA samples collected from L3PNs using laser capture microdissection. Moreover, to specifically study the L3PNs interconnecting DLPFC and PPC, we started using *in vivo* injections of fluorescent latex microspheres (retrobeads) to retrogradely label the PPC-projecting and DLPFC-projecting L3PNs in DLPFC and PPC. Our preliminary data show effective retrobead transport which allows us to identify the labeled L3PNs for electrophysiological, morphological and transcriptional analysis.

Disclosures: **G. Gonzalez-Burgos:** None. **T. Miyamae:** None. **D. Arion:** 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.; D.A.L. currently receives investigator-initiated research support from Pfizer. F. Consulting Fees (e.g., advisory boards); In 2012–2014 served as a consultant in the areas of target identification and validation and new compound development.

Poster

672. Modulation of Neuronal Firing Properties II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 672.18/C51

Topic: B.10. Intrinsic Membrane Properties

Support: U19 MH082441-06

Title: β-arrestin signaling increases excitability of fast-spiking interneurons in the prefrontal cortex

Authors: *S. GEE, P. O'DONNELL;
Pfizer, Cambridge, MA

Abstract: Activation of dopamine 2 receptors (D2R) not only activates downstream signaling pathways through canonical G-protein signaling, but also through non-canonical, β-arrestins. Indeed, all clinically effective antipsychotics interact with D2Rs and signal through both canonical and non-canonical pathways (Masri et al., 2008). Parsing apart the effects of these signaling pathways in prefrontal and striatal circuits may shine light on the mechanisms of current antipsychotics and lead to the development of more effective ones. Here, we examined the effects of second-generation antipsychotic, aripiprazole, and β-arrestin biased D2R ligand

UNC9994, on fast spiking interneurons (FSIs) in the prefrontal cortex. We performed whole-cell recordings from GFP-labeled FSIs in acute slices from GAD1-eGFP mice. We injected depolarizing current steps in current-clamp mode and recorded the number of action potentials generated. Aripiprazole elicited an increase in excitability in prefrontal FSIs, consistent with agonist-like activity previously reported with the D2R agonist, quinpirole (Tseng and O'Donnell, 2004). Interestingly, we found that UNC994 elicited a more robust increase in FSI excitability than the increase observed in aripiprazole. This effect was absent in FSIs recorded from β-arrestin2 KO mice, suggesting signaling through β-arrestin. Parvalbumin-positive FSIs in the prefrontal cortex are thought to be dysfunctional in schizophrenia and enhancing their activity may reverse the cognitive deficits observed in schizophrenia (Cho et al,. 2015). Thus, enhancing the activity of prefrontal FSIs by β-arrestin signaling may be an important mechanism of current antipsychotics and potentially relevant for the development of future ones.

Disclosures: **S. Gee:** A. Employment/Salary (full or part-time):; Pfizer. **P. O'Donnell:** None.

Poster

672. Modulation of Neuronal Firing Properties II

Location: Hall A

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Program#/Poster#: 672.19/C52

Topic: B.10. Intrinsic Membrane Properties

Title: Intrinsic plasticity during state-dependent calcium homeostasis in hippocampal model neurons

Authors: ***S. SRIKANTH**, R. NARAYANAN;
Indian Inst. of Science, Bangalore, Bangalore, India

Abstract: How do neurons reconcile the maintenance of calcium homeostasis with perpetual switches in afferent activity? Here, we assessed state-dependent evolution of calcium homeostasis in a population of hippocampal pyramidal neuron models, through an adaptation of a recent study on stomatogastric ganglion neurons (O'Leary, T. *et al*., Neuron, 2014). Calcium homeostasis was set to emerge through cell-autonomous updates to 12 ionic conductances, responding to different types of synaptically driven afferent activity. We first assessed the impact of theta-frequency inputs on the evolution of these conductances towards maintenance of calcium homeostasis. Although calcium homeostasis emerged efficaciously across all models in the population, disparate changes in ionic conductances that mediated this emergence resulted in variable plasticity to several intrinsic properties, also manifesting as significant differences in firing responses across models. Further, intrinsic neuronal properties and the firing response

were sensitive to the target calcium concentration and to the strength and frequency of afferent activity. Next, we studied the evolution of calcium homeostasis when afferent activity was switched between two behaviorally distinct types of activity: theta-frequency inputs and sharp-wave ripples. We found that the conductance values, intrinsic properties and firing response of neurons exhibited differential robustness to an intervening switch in the type of afferent activity. Finally, we asked how neurons that implement cell-autonomous calcium homeostasis react to knockout of specific ion channel conductances. To answer this, for each neuron, we removed specific conductances, one at a time, after steady state in calcium levels was attained while receiving theta frequency inputs. We assessed neuronal intrinsic properties at two time points: immediately following the knockout of the conductance (acute measurements) and at steady state in the post-knockout emergence of calcium homeostasis (through update of conductances other than the one that was knocked out) with theta-frequency inputs. We found that the robustness of models to acute knockouts and compensation-induced restoration of function were critically tied to specific neuronal measurements, with significant variability across measurements and across specific channels that were knocked out. These results unveil critical dissociations between different forms of homeostasis, and call for a systematic evaluation of the impact of state-dependent switches in afferent activity and genetic knockouts on neuronal intrinsic properties during neural coding and homeostasis.

Disclosures: **S. Srikanth:** None. **R. Narayanan:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Topic: B.10. Intrinsic Membrane Properties

Support: Conacyt-Mexico CB166241

Title: The biphasic effect of L-lactate on hippocampal cells spike frequency

Authors: *G. HERRERA-LOPEZ, E. J. GALVAN;
CINVESTAV SUR, Mexico City, Mexico

Abstract: Lactate has an important role as a supplemental energy substrate for the brain, but also work as a signaling molecule through the activation of the Gi protein-coupled receptor, HCA1 (HCAR1). Here, we sought to determine whether physiological concentrations of L-lactate modify the intrinsic excitability of hippocampal neurons. Whole cell recordings were performed

to assess the effects of L-lactate on the intrinsic properties and firing frequency of CA1 neurons. Perfusion of L-lactate (5 mM) reduced the input resistance and increased the rectification phase of the I-V curve as well the rheobase current to induce action potentials (n=8), indicating that L-lactate decreases the cellular excitability. Similar results were found with pyruvate (10 mM; n=9), but these effects were prevented when slices were preincubated with the lactate dehydrogenase inhibitor oxamate (20 mM; n=8). None of these parameters were modulated by the enantiomer D-lactate (5 mM; n=7). To explore whether lactate may act as a signaling molecule, the monocarboxilates transport was blocked with 4-CIN (0.5 mM; n=8). Under these conditions, L-lactate still reduced the intrinsic excitability of pyramidal cells, suggesting that the active transport of L-lactate is not responsible of the effects previously described. Next, we sought to determine whether the activation of the endogenous L-lactate receptor HCAR1 mimics the effects induced by L-lactate. The HCA1 receptor was activated with 3,5-dihydrobenzoic acid (3,5-DHBA, 0.56 mM, n=7), both passive properties and firing frequency exhibited similar changes as those induced by L-lactate, suggesting that lactate decreases the neuronal excitability of CA1 pyramidal cells through the activation of the HCA1 receptor. Interestingly, at lower concentrations of 3,5-DHBA (0.001 to 0.56 mM) or L-lactate (0.01 to 10 mM) pyramidal cells showed a reduction of the fire frequency. Conversely, higher concentrations of both molecules (1 mM to 10 mM for 3,5-DHBA and 20 to 30 mM for L-lactate) caused an increase in spike frequency. Lastly we examined the effects of L-lactate and 3,5-DHBA on the afterhyperpolarization current (IAHP). Lower concentrations of L-lactate increased the IAHP amplitude, but higher doses of 3,5-DHBA were required to increase the IAHP. The results suggest that activation of the HCA1R with L-lactate decreases neuronal excitability through a mechanism that involves upregulation of the IAHP.

Disclosures: **G. Herrera-Lopez:** None. **E.J. Galvan:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant NS047085

Title: Unitary synaptic connections among substantia nigra pars reticulata neurons

Authors: ***M. H. HIGGS**, C. J. WILSON;
Biol., The Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Substantia nigra pars reticulata (SNr) is a major basal ganglia output nucleus that integrates synaptic input from the direct and indirect pathways, combining this input with local inhibition via axon collateral synapses. Because SNr neurons fire rhythmically, these synapses are constantly active. To investigate their function, we recorded spontaneous IPSCs from SNr neurons in coronal brain slices, where afferents from upstream nuclei are severed. Most sIPSCs occurred at regular intervals and were tetrodotoxin-sensitive. Fourier analysis of sIPSC trains showed that each cell received 0-6 unitary inputs. Many cells had only one unitary input, providing an ideal preparation for synaptic studies. Presynaptic firing rates and inter-spike interval (ISI) variability inferred from sIPSC trains were similar to direct observations of SNr neuron firing. A running estimate of the phase of presynaptic firing was obtained by bandpass filtering the sIPSC trains, providing a statistical discrimination between unitary IPSCs (uIPSCs) clustered near the predicted spike times and miniature IPSCs that occurred randomly. We found that uIPSCs had large, variable amplitudes, fast kinetics, and synaptic failures. Based on failure rates, the mean quantal content ranged from 0.2-3 quanta. To estimate the synaptic release probability (p), variance-mean analysis of uIPSC amplitudes was performed using high-Ca^{2+} ACSF (4 mM Ca^{2+}, 0 Mg^{2+}) to increase p. In most cells, downward-curving variance-mean plots were obtained. On average, the data indicated that $p \sim 0.2$ in control ACSF (2 mM Ca^{2+}, 2 mM Mg^{2+}), suggesting that each unitary input makes 1-15 synapses. In combination with previous studies counting SNr axon collateral boutons, these data suggest that each cell forms and receives ~10-20 unitary inputs in the intact SNr. To investigate whether sIPSCs were depressed by autonomous activity, viral expression of halorhodopsin was used to silence presynaptic firing reversibly. The sIPSC amplitudes were not increased after silencing, suggesting that most synapses were not depressed. The synaptic reversal potential (E_{rev}) was estimated by perforated-patch current-clamp recording. Based on sIPSP detection rates and amplitudes across the ISIs, the mean E_{rev} was ~ -61 mV, which is slightly below the trough of the spike afterhyperpolarization. In part because of the change in driving force across the ISI, sIPSPs late in the ISI had the largest effect on spike timing. Our data show that local axon collateral synapses provide strong, fast inhibition that perturbs SNr neuron spike timing, consistent with the hypothesis that these connections act to desynchronize the basal ganglia output.

Disclosures: **M.H. Higgs:** None. **C.J. Wilson:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant NS072197

Title: The mechanism for beta frequency membrane resonance and its effect on spiking in striatal LTS interneurons

Authors: *S. C. SONG[1], J. A. BEATTY[2], C. J. WILSON[1];
[1]Biol., Univ. of Texas At San Antonio, San Antonio, TX; [2]Physiol., Michigan State Univ., East Lancing, MI

Abstract: Striatal interneurons exhibit membrane resonances, each in a cell-type specific frequency band. Somatostatin- and NPY-positive low threshold spike (LTS) interneurons have their membrane resonance in the beta frequency range. The membrane resonance is tetrodotoxin insensitive but cadmium sensitive. We sought to determine the specific ion channels that produce the beta frequency membrane resonance in LTS interneurons. We also investigated the influence of membrane resonance on the neurons proclivity to phase-lock their action potentials to beta-frequency components of complex input waveforms (spiking resonance). To investigate both the mechanism for membrane resonance and its effect on spiking resonance, perforated-patch recordings were made in mouse striatal slices. The mice expressed GFP under the control of the NPY promoter to visually identify LTS cells in slice. To determine the ion channels responsible for the membrane resonance, voltage clamp frequency sweeps were applied to the LTS interneurons to measure its impedance at a range of input frequencies. The frequency sweeps were applied in control, ACSF conditions as well as in the presence of specific ion channel blockers. We found that blockade of N-type (CaV2.2) calcium channels (but not L or P/Q channels) specifically blocked membrane resonance. Blockade of calcium-activated chloride channels (CaCC) with niflumic acid was equally capable at preventing membrane resonance. We suggest a resonance mechanism for LTS cells in which depolarization activates CaV2.2 calcium current, which specifically activates CaCCs. To measure the contribution of membrane resonance to spiking resonance, shot-noise simulated EPSPs, designed to equally represent synaptic inputs at a range of frequencies, were applied to the neuron. The phase with which spikes occurred in regards to the Fourier filtered input was measured to determine the spiking resonance frequency. This was performed in control, ACSF conditions as well as with ω-conotoxin GVIA or niflumic acid present. The spiking resonance profile changed in the presence of the membrane resonance blockers, suggesting influence of membrane resonance on frequency specific phase-locking of striatal LTS interneurons to beta frequency components in their input.

Disclosures: **S.C. Song:** None. **J.A. Beatty:** None. **C.J. Wilson:** None.

Poster

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Topic: B.10. Intrinsic Membrane Properties

Support: NICHD R15HD075207

Title: Prolonged spinal networks activation induces adaptive alterations in spinal motoneuron intrinsic excitability

Authors: *J. LOMBARDO, M. HARRINGTON;
Delaware State Univ., Dover, DE

Abstract: The persistent modification of neuronal properties resulting from past experiences and affecting future behavior has been presumed to exclusively involve the brain. However, recent work indicates that activity-dependent plasticity occurs in spinal motor neurons during development, as well as later in life with skills acquisition and maintenance, and in response to trauma and disease. Understanding how spinal motoneuron output can be modified by both increased and decreased activity is thus a fundamental challenge with implications for athletic training, rehabilitation and advanced prosthetics. To characterize the activity-dependent modulation of spinal motoneuron intrinsic properties, we have treated spinal cord slices for about 30 minutes with different stimulants that have been shown to trigger patterns of locomotion-like activity in spinal cord preparations. The intrinsic excitability of spinal motoneurons in treated slices was significantly altered; in particular, the resting membrane potential was hyperpolarized, input resistance was reduced and current threshold was increased. While other interventions can alter spinal motoneuron intrinsic properties, the changes triggered by sustained ~30 min-long locomotion-like-triggered spinal network activation have a unique time course - different from both central fatigue which is observed over seconds to minutes, and training-induced plasticity which emerges over days to weeks. Realistic conductance-based computational models of spinal motoneurons allowed us to identify potential mechanisms underlying the changes in intrinsic properties. Pharmacological tools were then used to test whether the mechanisms identified in silico were indeed occurring *in vivo*, and to identify intracellular signaling pathways that alter spinal motoneuron intrinsic excitability after prolonged spinal network activation.

Disclosures: J. Lombardo: None. **M. Harrington:** None.

Poster

672. Modulation of Neuronal Firing Properties II

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Topic: B.10. Intrinsic Membrane Properties

Title: Chemogenetic control of the activity of cholinergic interneurons in the striatum

Authors: *S. CHOI[1,2], Y. DING[2], E. V. MOSHAROV[2], U. KANG[2];
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Abstract: Striatal cholinergic interneurons (ChI) are implicated in motor control, associative plasticity, and reward-dependent learning. ChIs exhibit tonic action potential firing which consist of a brief (200-300 ms) cessation of firing, termed the pause response, and, depending on the nature of the stimulus and its behavioral context, suggesting a prominent role in plasticity. The critical question is how gain or loss of cholinergic activity regulate the functioning of striatum. Recent studies have shown that brief train stimulation by optical light on the ChI generates inhibitory responses in the medium spiny neurons (MSN), principal cells of striatum through increasing GABAergic conductance. We investigated pharmacological control of ChI by using chemogenetics and patch clamp recording in brain slices. Virally introduced Designer Receptors Exclusively Activated by Designer Drugs (DREADD) under choline acetyltransferase (ChAT) promotor is activated by CNO which either increase or suppress ChI activity through hM3Dq (Gq or Gs-coupled) and hM4Di (Gi/o-coupled) respectively. We found that electrophysiological membrane properties (i.e. resting membrane potential, I/V relationship, and input resistance) which were measured from hM3Dq, hM4Di, and control animal showed no difference between groups. 1 μM CNO application for 5 minutes on hM3Dq expressed ChI successfully increased tonic activity of neurons but it was not recovered after CNO washout. However, 10 μM CNO reversely suppressed tonic firing of hM4Di expressed ChI. These results suggest that chemogenetics is a feasible approach to control tonic cholinergic activity and this will help to explore unknown circuit mechanism between ChI and other principal neurons in the striatum.

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Poster

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ERC AdG 250345

Title: Inverse stochastic resonance in cerebellar Purkinje cells

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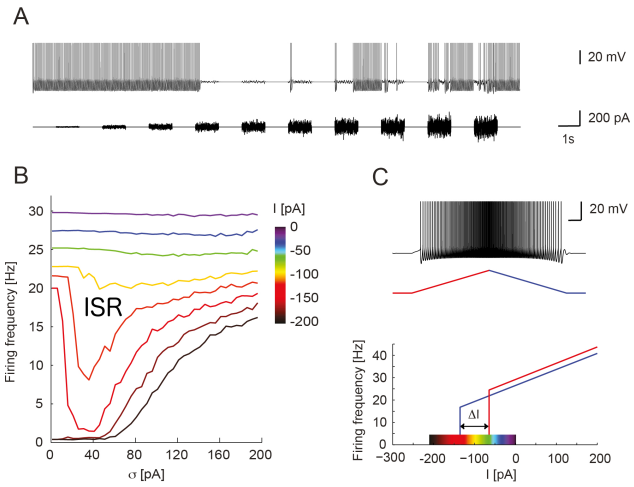
Abstract: Purkinje cells play an important role in cerebellar computation since their axons are the only projection from the cerebellar cortex to deeper cerebellar structures. They have complex internal dynamics, which allow them to fire spontaneously, display bistability and participate in network phenomena such as high frequency oscillations and travelling waves. Purkinje cells have type II excitability, which can be revealed as a discontinuity in their f-I curves. We show that this excitability mechanism allows Purkinje cell simple spiking to be efficiently inhibited by noisy current input of a particular variance, a phenomenon known as Inverse Stochastic Resonance (ISR). While this effect has been observed previously in theoretical models of single neurons, here we provide the first experimental demonstration of ISR. We find that an adaptive exponential integrate-and-fire model (aEIF), fitted using a modified dynamic IV method to reproduce the basic intrinsic properties of Purkinje cells, displays ISR and bistability between the rest state and a limit cycle representing repetitive activity. We then use the aEIF model to explain the link between the ISR and bistability in Purkinje cells. We show that ISR allows Pukinje cells to operate in different functional regimes, from the all-or-none toggle to the linear filter mode, depending on the variance of the background synaptic input. We propose that changes in synaptic noise provided by parallel fiber input allow Purkinje cells to quickly switch between these functional regimes. Using mutual information analysis, we demonstrate that ISR leads to an optimum in information transfer between the input and output spike train of the Purkinje cell. These results provide the first experimental evidence for ISR and suggest possible functional roles for ISR in cerebellar information processing.



Disclosures: **A. Buchin:** None. **S. Rieubland:** None. **M. Hausser:** None. **A. Roth:** None. **B. Gutkin:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.01/C59

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DIRP, NIMH

Title: Memory, activity, olfaction, and sleep in a transgenic rat model of Alzheimer’s disease

Authors: L. KRYCH[1], R. REITH[1], A. K. SMITH[2], R. M. COHEN[2], *C. B. SMITH[1];
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Abstract: Alzheimer’s disease (AD) is a chronic neurodegenerative disease characterized by debilitating cognitive decline. The hallmark pathological changes in AD are extracellular deposition of the amyloid-beta peptide, intracellular inclusions of hyperphosphorylated microtubule-associated protein tau, aggravated neuroinflammation, and neuronal loss. The rat transgenic model of AD (TgF344-AD) recapitulates these four age-dependent characteristics of the disease and is accompanied by indications of declining memory function (Cohen et al. (2013) J Neurosci 33:6245). In the present study, we further investigated behavioral changes in TgF344-AD rats. We measured the following behaviors in both male and female WT and TgF344-AD

rats: olfaction (buried food task), activity in a novel environment (open field test), and hippocampal-dependent learning and memory (rewarded alternation in the T-maze). We report preliminary results in TgF344-AD and WT rats studied at 6 months (22 TgF344-AD, 22 WT) and 12 months (11 TgF344-AD, 9 WT) of age. Results of the buried food task indicate no olfactory impairment at either age (two sample t-tests). In the open field, TgF344-AD rats at 6 months of age were less active than WT (RM ANOVA, main effect of genotype, $p < 0.001$), whereas at 12 months of age differences did not reach statistical significance. Performance in the T-maze was similar in the two genotypes at 6 months of age, but at 12 months of age performance of TgF344-AD rats suggests a learning and memory deficit (2-sample test for equality of proportions, $p < 0.001$). In a subset of animals 6 months of age, we studied sleep patterns by means of an infrared home-cage monitoring system. Male rats demonstrate a genotype-dependent difference in total sleep time (6 TgF344-AD, 9 WT) (two sample t-tests, $p < 0.05$), but genotype-dependent differences in total sleep time were not observed in females (9 TgF344-AD, 6 WT). These observations of changes in sleep patterns call for further investigation. Taken together our preliminary results confirm the learning and memory deficit manifesting around 12 months of age in the TgF344-AD rat model.

Disclosures: **L. Krych:** None. **R. Reith:** None. **A.K. Smith:** None. **R.M. Cohen:** None. **C.B. Smith:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

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Program#/Poster#: 673.02/C60

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Office of Research and Sponsored Programs at CMU (to TH)

Field Neurosciences Institute (to GD)

John G. Kulhavi Professorship (to GD)

Central Michigan College of Medicine (to JR)

Title: Alzheimer's disease early pathology and behavioral characterization in male and female 5xFAD mouse model of Alzheimer's disease

Authors: ***T. HALL**[1,2], C. LEARMAN[1,2], E. BATES[1,2], L. PALADUGU[1,2], M.-S. SONG[1,5], J. ROSSIGNOL[1,2,3], G. DUNBAR[1,2,4,5];

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Abstract: Alzheimer's disease (AD) is an intricate protein misfolding neurodegenerative disorder characterized by profound memory loss and a neuropathological profile that includes: inflammation, oxidative stress, neurofibrillary tangles, and amyloid-β (Aβ) deposits; all of which contribute to neuronal dysfunction and atrophy. There are myriad of risk factors associated with developing AD, with aging being the number one factor. However, gender is also a risk factor of concern, with the proportion of women diagnosed with AD outnumbering men. Aβ plays a crucial role in AD pathology, much of the current research has focused on the extracellular Aβ plaque deposits. However, within the past decade it has been shown that the more toxic species is the oligomeric Aβ fragments (oAβ), largely due to its abilities to be transmitted from cell-to-cell and misfold other like proteins. With advances in AD early detection methods via various biomarkers within the cerebral spinal fluid (CSF), and the current emergence of oAβ positron emission tomography (PET) scans, the possibility of new treatment therapies centered on early interventions is plausible. Current research focuses on understanding the underlying mechanisms behind this "seeding" phenomenon to possibly provide a pathological timeline with key target locations for early treatment and intervention. The aim of the present study was to asses when and where certain AD-like pathologies emerge in both male and female 5xFAD transgenic mice at the ages of 1,2, and 4 month of age. Levels of various Aβ species and associated signaling molecules were assessed in brain in brain samples and blood serum via western blot and immunohistological assays. An array of behavioral batteries were utilized to assess anxiety, motoric and cognitive abilities, including: open field, novel object recognition, passive avoidance, and clasping. This study is the first to specifically compare early pathology with behavior between males and females in 5xFAD mice.

Disclosures: **T. Hall:** None. **C. Learman:** None. **E. Bates:** None. **L. Paladugu:** None. **M. Song:** None. **J. Rossignol:** None. **G. Dunbar:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.03/C61

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: High-throughput phenotyping of transgenic AD models

Authors: *D. BRUNNER, T. HANANIA, M. MAZZELLA, H. HAIN, E. SABATH, V. ALEXANDROV, J. BERGER, P. KABITZKE, K. COX, M. WINDISCH;
Psychogenics Inc, Tarrytown, NY

Abstract: Three popular models for Alzheimer's disease were investigated using behavioral high-throughput phenotyping technologies: : the single transgenic Tg 2576 (mutant APP) mouse model, the double transgenic model from the cross between the Tg 2576 and a mutant presenilin (PS1) line both expressing increased amyloid- beta 40 and 42 levels and amyloid pathology, and the rTg4510 mice, overexpressing human tau with the P301L mutation under control of a tetracycline responsive transacting element, showing cognitive impairment, motor deficits, tau hyperphosphorylation, neurofibrillary tangles, and neuronal loss in the forebrain. We exploited the option to switch off tau expression by doxycycline in the Tg4510 model, as a calibration for maximal effect of a putative treatment. PhenoCube® NeuroCube® and SmartCube® are high-throughput platforms that assess circadian, cognitive, motor behavior, anxiety, gait, and other domains using PGI's proprietary Computer Vision automated scoring system and machine learning algorithms to define phenotypic signatures. APP/PS1 mice were hyperactive as early as 12 weeks of age as assessed in our high-throughput phenotypic platforms. Tg2576 mice showed a milder yet similar signature. The deficits in the Tg4510 were age-dependent unlike those of the tTa control, which showed a significant phenotype at the earlier ages. Doxycycline partially reversed the Tau behavioral signature. The possibility to evaluate behavioral deficits with the novel high-throughput technology early in life forms a basis to test therapeutic interventions at early stages of developing brain pathology.

Disclosures: D. Brunner: A. Employment/Salary (full or part-time):; PsychoGenics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsychoGenics. **T. Hanania:** A. Employment/Salary (full or part-time):; PsychoGenics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsychoGenics. **M. Mazzella:** A. Employment/Salary (full or part-time):; PsychoGenics. **H. Hain:** A. Employment/Salary (full or part-time):; PsychoGenics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsychoGenics. **E. Sabath:** A. Employment/Salary (full or part-time):; PsychoGenics. **V. Alexandrov:** A. Employment/Salary (full or part-time):; PsychoGenics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsychoGenics. **J. Berger:** A. Employment/Salary (full or part-time):; PsychoGenics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsychoGenics. **P. Kabitzke:** A. Employment/Salary (full or part-time):; PsychoGenics. **K. Cox:** A. Employment/Salary (full or part-time):; psychogenics. **M. Windisch:** A. Employment/Salary (full or part-time):; psychogenics.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.04/C62

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

CCNA

Title: Assessing outcomes of a middle cerebral artery occlusion injury in an APP transgenic rat

Authors: *A. M. REGIS[1], V. HACHINSKI[1,2], S. N. WHITEHEAD[1,2];
[1]Anat. & Cell Biol., [2]Clin. & Neurolog. Sci., Western Univ., London, ON, Canada

Abstract: It is estimated that by age 60, one in three individuals will develop Alzheimer's disease (AD), suffer a stroke, or experience both. While traditionally regarded and treated as distinct conditions, clinical and experimental findings begin to suggest an interaction may occur. However, conclusive pathophysiological links have yet to be determined. Based upon past studies, it may be hypothesized that following ischemic stroke, a transgenic rat model carrying human APP mutations will display enhanced Aβ deposition, suffer more severe cognitive decline and display increased stroke-related pathology. To model AD pathology, our study uses a genetically engineered rat model exhibiting mutations in the amyloid precursor protein (APP); this model overproduces the human pathogenic protein attributable in AD called amyloid-β. To model stroke injury, a macrosphere-induced permanent Middle Cerebral Artery occlusion (pMCAo) strategy will be utilized. pMCAO provides a clinically relevant model of human embolic ischemic stroke of the middle cerebral artery, a common type of human ischemic stroke. The proposed models will be studied both in combination as well as singularly over a period of two months post-stroke. A variety of behavioural tests will be used to determine any apparent cognitive and motor deficits, while biochemical analytical methods will be used to determine the mechanistic changes associated. These strategies will help us better understand how stroke and AD manifest themselves in combination throughout the disease processes. Preliminary results from our laboratory using rat models suggest that in the presence of amyloid deposition, facets of stroke including infarct size and the degree of neuroinflammation are heightened. These pathological effects are shown to be translated into behavioural deterioration in rodent models of AD and stroke. However, further studies are required to truly understand the interactions between amyloid toxicity, ischemic stroke damage, and neuroinflammation. Upon defining the specific pathological mechanisms, use of therapeutic intervention may be employed. Overall,

with a better understanding of the mechanistic roles of stoke and AD pathology on resultant cognitive impairment, it may be possible to employ strategies in rodents to minimize post-stroke cognitive burden; interventions that may become a translational strategy in humans who are susceptible to stroke and Alzheimer's disease related cognitive impairment.

Disclosures: A.M. Regis: None. **V. Hachinski:** None. **S.N. Whitehead:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.05/C63

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NIA PO1 AG00538

Alzheimer's Association IIRG-11-204835

Title: Increased cellular senescence in mouse models of Alzheimer's disease (or amyloidosis)

Authors: *G. F. PASSOS[1,2], R. DA COSTA[1], R. MEDEIROS[2], D. H. CRIBBS[2]; [1]Pharmaceut. Biotech., UFRJ, Rio de Janeiro, Brazil; [2]Inst. for Memory Impairments and Neurolog. Disorders, Univ. of California, Irvine, Irvine, CA

Abstract: Advanced age is considered the major risk factor for Alzheimer's disease (AD), however, the mechanisms underlying the contribution of aging to the development of AD are largely unknown. There is increasing evidence for a role of cellular senescence in physiological aging but also in injury and disease. Nevertheless, the mechanisms driving cellular senescence in the brain as well as how senescent cells affect brain pathology remain unclear. In this study, we investigated the molecular mechanisms associated with cellular senescence in AD. In order to determine whether amyloid-beta (Aß) accumulation, a pathological hallmark of AD, is associated with senescence *in vivo*, brain tissue from two different transgenic models of the disease were examined for the presence of increased senescence-associated beta-galactosidase (SA ß-gal) activity, a marker of senescence. Compared with age-matched nontransgenic (nTg) mice, a significant increase in SA ß-gal activity was observed in cortex and hippocampus of 24-month-old Tg2576 mice. Likewise, SA ß-gal activity was found to be significantly increased in the thalamus of TgSwDI mice, compared to nTg mice, when evaluated at 9 months of age. Taken together, our results suggest that the presence of senescent cells may contribute to the

pathogenesis of AD and may represent a link between the aging process and progression of the disease.

Disclosures: **G.F. Passos:** None. **R. da Costa:** None. **R. Medeiros:** None. **D.H. Cribbs:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.06/C64

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Sponsor donation

J. Yang and Family Foundation.

Title: Hindlimb locomotion pattern changes are detected in both the intact and spinalized Alzheimer's disease model J20 mice

Authors: ***R. HUANG**[1,2], M. S. JOSEPH[1,3], R. R. ROY[1,3,4], H. ZHONG[1,3,4], E. H. KOO[5], R. V. EDGERTON[1,3,4], D. C. LU[2,5,1,4];
[2]Dept. of Neurosurg., [3]Dept. of intergrative Biol. and Physiol, [4]Brain Res. Inst., [1]UCLA, Los Angeles, CA; [5]Dept. of Neurosciences, UCSD, San Diego, CA

Abstract: Alzheimer's Disease (AD) is devastating and costly. AD is characterized by progressive loss of memory and other cognitive impairments. Although often overlooked, motor impairments with gait and postural stability are a common feature of AD. These deficits are generally considered secondary impairments, attributed to cerebral degeneration and dementia, but detailed studies of spinal cord function in AD are lacking. To study the potential role of spinal cord involvement in AD, we used an AD mouse model (PDGF-APPswe/Ind mice (J20)) and characterized changes in stepping assays as well as spinal Amyloid-precursor Protein (APP)/ Amyloid-beta (Aβ) expression in intact and spinally transected animals. We used the treadmill stepping paradigm to evaluate locomotion kinematics in J20 mice and compared them to wild-type (WT) littermates. Both J20 and WT mice were also divided into 4-month and 13-month groups to investigate the influence of AD-like pathological progression on stepping pattern (13-month J20, n=13; 13-month WT, n=10; 4-month J20, n=7; 4-month WT, n=7). The treadmill stepping was analyzed by the SIMI motion capture system for all groups. We found that J20 mice in both age groups exhibited locomotion difficulties when compared to their WT littermates. Our ELISA results showed an Aβ increase in all spinal cord segments of both 4-

month and 13-month J20 mice. To further investigate how spinal circuitry might contribute to motor alternations in J20 mice, complete spinal cord transections at the mid-thoracic level (Thoracic 7-9) was performed to isolate lumbo-sacral spinal circuitry (J20, n=7; WT, n=7). After a 2-week post-surgery recovery period, hindlimb locomotion of the spinalized mice was tested with the bipedal treadmill stepping paradigm before and after quipazine delivery for 6 weeks. We found J20 spinal mice recovered slower than WT controls but quipazine induced more bipedal treadmill steps in J20 spinal mice relative to WT controls. These results suggest differences in the functional output of J20 and WT lumbosacral spinal cord. The treadmill stepping together with the molecular results suggest that early and potentially independent changes in spinal cord circuitry are present in J20 mice. Changes in recovery time and in the response to serotonin agonist observed after spinal transection further support the hypothesis that motor behavior in J20 mouse after spinal transection can be attributed at least in part to dysfunctions caused by Aβ within the sensory-motor networks of the lumbosacral spinal cord segments.

Disclosures: **R. Huang:** None. **M.S. Joseph:** None. **R.R. Roy:** None. **H. Zhong:** None. **E.H. Koo:** None. **R.V. Edgerton:** None. **D.C. Lu:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.07/C65

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC of Canada

Title: Age-related changes in feeding behaviours, weight, hormones, and metabolism in 12-month old female 5xFAD mice

Authors: ***W. H. GENDRON**[1], R. E. BROWN[1], S. PELLETIER[1], Y. ANINI[2];
[1]Psychology, [2]Physiol. and biophysics, Dalhousie, Halifax, NS, Canada

Abstract: The 5xFAD mouse is a double transgenic model of Alzheimer's disease (AD), which carries an amyloid precursor protein (APP) transgene with three mutations, and a presenilin-1 transgene with two mutations. These mutant transgenes act additively to produce increases in beta amyloid (AB)-peptides, and the presence of AB-plaques emerges at two months of age. Weight-loss is an issue in human AD patients, and age-related weight-loss is also seen in 5xFAD mice. We therefore investigated age-related changes in body weight, feeding behaviour, activity

levels, feeding-related hormones, and leptin and hormone sensitive lipase mRNA in female 5xFAD mice and their WT (C57BL/6JxSJL/J F1) controls from 3 to 12 months of age. The 5xFAD mice weighed more than WT controls at 9 and 12 months of age ($p<0.05$), but no differences were found in food intake between genotypes except at 9 and 12 months of age WT ate more than 5xFAD mice ($p<0.05$) . Levels of grooming and jumping did not differ between genotypes ($p>0.05$), however, 5xFAD mice showed less climbing and rearing ($p<0.05$), and spent more time remaining still than WT mice ($p<0.05$). At 12 months of age there were no genotype differences in plasma insulin or glucose concentrations ($p>0.05$). Perigonadal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) were collected and it was found that 5xFAD mice had less WAT than WT mice ($p<0.05$). No differences in BAT were seen. Expression of mRNA for leptin and hormone sensitive lipase was measured in WAT, and the 5xFAD mice expressed reduced levels of both mRNA's ($p<0.05$). Muscle tissue was collected to evaluate mice for sarcopenia (muscle loss), but the 5xFAD mice did not differ in muscle mass compared to WT mice ($p>0.05$). Our data suggest that weight-loss seen in 5xFAD may be partially explained by reduced food intake. We also conclude that the 5xFAD mice are hypoactive compared to WT behaviours that require a significant amount of energy. The 5xFAD mouse model does not suffer from sarcopenia, indicating that all motor deficits are likely due to neural defects. Finally the 5xFAD mice have less fat and express less leptin and hormone sensitive lipase mRNA compared to WT mice, indicating that they have less leptin and that fat loss is the main contributor to weight-loss in this mouse model.

Disclosures: W.H. Gendron: None. **R.E. Brown:** None. **S. Pelletier:** None. **Y. Anini:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.08/C66

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 15-BD-0402

Title: Peripheral nervous system is vulnerable before significant development of pathology in Alzheimer's disease mouse model

Authors: *J. KIM, A. A. B. RASHEED, S.-J. YOO, C. MOON;
DGIST, Dae gu, Korea, Republic of

Abstract: Abnormal processing of amyloid precursor protein (APP), through sequential cleavages first by β-secretase and then by γ-secretase complex, leads to excessive production of β-amyloid (Aβ) in the central nervous system (CNS), that is Alzheimer's disease. Specially, γ-secretase complex have presenilin1 and 2 as the catalytic core and that's gene mutations markedly trigger Aβ accumulation. Olfactory epithelium (OE), a kind of peripheral nervous system, could send the information from the brain to the external environment. Before AD symptoms such as memory loss or muscle retardation turn up, olfactory dysfunction usually occurs. It means that OE, odorant first detection site, have some problems. But it is unknown well the correlation of olfactory deficit and AD. Here, we found that 10month TG2576 mice meaning the early stage of AD have olfactory dysfunction. And APP processing and Presenilin2 expression pattern are differ in PNS and CNS. Specially, PS2 levels of TG2575 mice are increased significantly in mRNA and protein of PNS, compared with CNS. Through these results, there are differences between mechanisms of development of AD of PNS and CNS. It might help to detect the AD before the progression through the PNS, indirectly but easily.

Disclosures: J. Kim: None. **A.A.B. Rasheed:** None. **S. Yoo:** None. **C. Moon:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.09/C67

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Scottish Rite Charitable Foundation

Title: Aerobic glycolysis in the frontal cortex correlates with memory performance in wild-type but not APP/PS1 mice: implications for metabolic intervention in Alzheimer's disease

Authors: *R. A. HARRIS[1], R. CUMMING[2];
[1]Biol., [2]Univ. of Western Ontario, London, ON, Canada

Abstract: The majority of glucose consumed by the adult brain is fully oxidized in the mitochondria of neurons to supply the large amounts of ATP required for synaptic transmission. However, a certain percentage of glucose in the brain is exclusively metabolized by glycolysis to generate lactate, even when oxygen is not rate limiting. This form of metabolism is known as aerobic glycolysis. Emerging evidence now suggests that aerobic glycolysis in the brain plays a critical role in generating biosynthetic metabolites during early CNS development and persists in certain regions of the adult brain to support synaptic plasticity, learning and memory. However,

aerobic glycolysis steadily declines with age and virtually disappears in the elderly. Our lab has recently demonstrated that a metabolic shift to aerobic glycolysis confers nerve cells with a survival advantage against the toxic effects of amyloid beta, a key pathogenic peptide in Alzheimer's disease (AD). However, the beneficial effect of aerobic glycolysis in the AD brain remains to be fully elucidated. In this study, we demonstrate that a progressive decline in aerobic glycolysis occurs in the mouse brain with age, which correlates with a loss of spatial learning and memory in APP/PS1 mice (AD mice). Proton magnetic resonance spectroscopy revealed an age-dependent decline of lactate levels in the frontal cortex of control mice but not in AD mice. Western blot analysis of extracts from the frontal cortex revealed an age-dependent decline in key regulatory proteins of aerobic glycolysis in both control and AD mice at 12 months old. In contrast, lactate transporter expression increased with age. The expression of aerobic glycolysis enzymes correlated with better memory performance in the Morris Water Maze for control mice, but not AD mice. Confocal microscopy revealed a high expression of aerobic glycolysis enzymes in astrocytes surrounding plaques in the cortex of AD mice. Aerobic glycolysis enzymes were also expressed in the cell bodies of Tuj1 neurons in both control and AD mice. These data indicate that aerobic glycolysis and the production of lactate in the brain naturally declines with age, yet is compensated by up-regulation of lactate transporters. However, this process appears to be interrupted by the presence of amyloid plaque in the AD brain and may play a role in memory decline. This study suggests that metabolic intervention may be a therapeutic strategy for the treatment of memory loss in AD.

Disclosures: R.A. Harris: None. **R. Cumming:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.10/C68

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Young to middle-aged dogs with high Abeta-levels in CSF are impaired on learning in standard cognition tests.

Authors: *H. BORGHYS[1], D. DHUYVETTER[2], B. VAN BROECK[3], J. ARAUJO[4], M. BROOKS[5];
[1]Janssen Res. & Develop., Beerse, Belgium; [2]janssen, Beerse, Belgium; [3]Janssen, Beerse, Belgium; [4]InterVivo, Toronto, ON, Canada; [5]Intervivo, Toronto, ON, Canada

Abstract: Understanding the relevance of changes in Alzheimer's disease biomarkers that occur before the pathology becomes evident, can contribute to the development of a treatment for

Alzheimer's disease. A longitudinal follow-up of an animal species with a similar amyloid pathology in the brains as in humans may contribute to this research.Amyloid plaque formation is one of the two main neuropathological hallmarks of Alzheimer's disease in humans. Dogs are similar to man with respect to amyloid precursor protein (APP)-processing and age-related amyloid plaque deposition. Dogs also are used as a natural model of age-dependent cognitive dysfunction. In our colony of beagle dogs Aβ-concentrations in cerebrospinal fluid (CSF), sampled in awake animals from the lateral ventricle, were regularly measured over a period of years. We identified dogs showing low or high Aβ42 levels and formed two groups of ten animals each. The age of the animals, which ranged from 2-8 years, was comparable between both groups. Since dogs normally start to develop amyloid plaques from an age of 9-10 years onwards, these dogs are assumed to have no or minimal amyloid plaque formation. The cognitive performance of these dogs was evaluated in standard cognition tests such as object discrimination learning, reversal learning and delayed non-match to position (DNMP). A difference in learning performance was observed between dogs with low and high CSF Aβ concentrations. Our data suggest that high levels of Aβ in young to middle-aged dogs might contribute to learning impairment prior to amyloid deposition. Further experiments are needed to investigate whether there is a causal link between high levels of CSF Aβ and cognitive performance in young to middle-aged dogs as well as the longitudinal sequalae of these differences with respect to disease progression.

Disclosures: **H. Borghys:** A. Employment/Salary (full or part-time):; Janssen. **D. Dhuyvetter:** A. Employment/Salary (full or part-time):; Janssen. **B. Van Broeck:** A. Employment/Salary (full or part-time):; Janssen. **J. Araujo:** A. Employment/Salary (full or part-time):; InterVivo. **M. Brooks:** A. Employment/Salary (full or part-time):; InterVivo.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

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Program#/Poster#: 673.11/C69

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 514219 6GMH F86 ARUK Wiseman

Title: Breaking Down Alzheimer's disease in Trisomy 21

Authors: ***L. PULFORD**[1], M. RICKMAN[1], S. NOY[1], J. TOSH[1], D. ABUCEWICZ[2], V. L. J. TYBULEWICZ[2], E. M. C. FISHER[1], F. K. WISEMAN[1];
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Abstract: Down syndrome (DS) or Trisomy 21, caused by inheriting an extra copy of human chromosome 21 (Hsa21), leads to a greatly elevated risk of developing Alzheimer's disease. This is largely due to triplication of the amyloid precursor protein gene (APP), present on Hsa21. APP is processed to produce Aβ, a toxic species central to AD pathogenesis. Despite nearly universal presence of AD plaques and tangles by 40 years, not all individuals with DS go on to develop dementia, and the age of onset varies. Alongside more aggressive pathology, individuals with AD/DS also have an elevated incidence of seizures compared to sporadic AD. With over 230 protein-coding genes on Hsa21 it is likely that genes other than APP contribute to, or even protect against the development of dementia in DS. The transchromosomic (Tc1) mouse model of DS contains a freely segregating copy of Hsa21 and is functionally trisomic for around 75% of Hsa21 genes. Importantly, the Tc1 mouse is not functionally trisomic for APP. When crossed with the J20 (APPSwInd) model of APP/Aβ pathology, Tc1 trisomy resulted in an exacerbation of Aβ plaque load, a sensitisation to APP/Aβ-induced behavioural deficits and a reduction in survival. This suggests that Hsa21 genes other than APP lead to an exacerbation of AD pathogenesis in DS. Chromosomal engineering has allowed us to 'fragment' Hsa21 in order to map the genetic region responsible for this exacerbation. To look at the contribution of 40 genes on mouse chromosome 10 (Mmu10) that are syntenic with Hsa21, we have similarly crossed a partial trisomy model (the Ts2Yey mouse) to the J20 mouse. In contrast to the Tc1 x J20 cross, Ts2Yey trisomy rescues the sudden-death phenotype observed in J20 mice, which is thought to be caused by epileptiform activity. This is coupled with a reduction in astrogliosis at 6 months, however no change in soluble or insoluble Aβ levels, Aβ plaque load, full-length APP protein or its C-terminal fragments is observed. To investigate whether the rescue of the sudden-death phenotype in Ts2Yey;J20 mice is due to a normalisation of hypersynchronous network activity, an electroencephalogram (EEG) study is underway. Two novel mouse crosses used here demonstrate the complexity of AD pathogenesis in DS, and aid in explaining the heterogeneity of dementia onset in the DS population. The Ts2Yey region of Hsa21 may confer some level of protection against AD-related phenotypes and further work is required to uncover the mechanism behind this result. Analysis of additional partial trisomy models of DS will allow the dissection of the region, and the genes, contributing to the aggravation of AD pathology in DS and the wider population.

Disclosures: **L. Pulford:** A. Employment/Salary (full or part-time):; Alzheimer's Research UK. **M. Rickman:** None. **S. Noy:** None. **J. Tosh:** None. **D. Abucewicz:** None. **V.L.J. Tybulewicz:** None. **E.M.C. Fisher:** None. **F.K. Wiseman:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.12/C70

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant EKA1R01NS0

Title: A graph diffusion model predicts a primarily retrograde pattern of pathology propagation in mouse models of Alzheimer's disease

Authors: ***C. MEZIAS**, E. LOCASTRO, A. RAJ;
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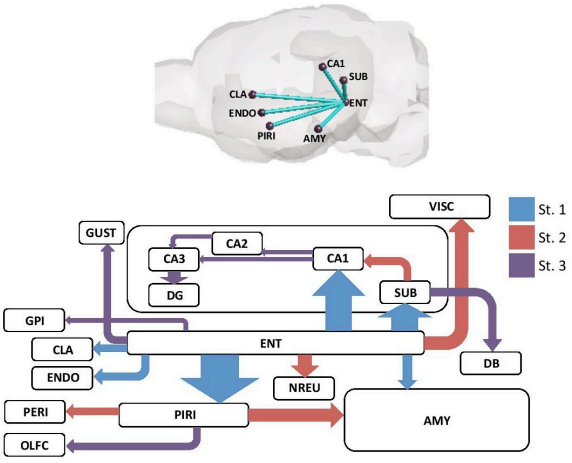
Abstract: Much of the research into Alzheimer's Disease (AD) has focused on the proximal cause of cognitive decline, neuronal loss, and protein pathologies. However, AD is not a disorder that simply stays put or seeds in multiple areas. Rather, the protein pathology hallmarks of the disease and the neuronal damage progress throughout the brain and are mirrored by the progressive cognitive deficits associated with the disease and an area of intense current research is the directional preference of the spread of these pathologies. Here we posit a model that explains the spread of pathological proteins, tau and amyloid, and neuronal loss solely based on the directional connectivity network of both the hippocampus, and the entire brain. Comparing the predictions of the model to small datasets of both tau and amyloid burden and neuronal loss in different sub-regions of the hippocampus and regions of the brain, we see an early APP and amyloid spread, and later stage tau proliferation primarily occurring in the retrograde direction, as well as a slight preference for cell loss pathology to proceed in this direction. Taken together, our results show that pathology in AD has a preference to spread in a primarily retrograde manner.



Disclosures: **C. Mezias:** None. **E. LoCastro:** None. **A. Raj:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.13/C71

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Brain Research Trust 4-year PhD programme in Clinical Neuroscience

Title: How does Trisomy 21 in Down syndrome exacerbate Alzheimer's disease pathology in a novel mouse model, Tc1xJ20?

Authors: *X. CHOONG[1,3], M. ZANDA[2,4], V. PLAGNOL[2], V. L. J. TYBULEWICZ[5,3], F. K. WISEMAN[1,3], E. M. C. FISHER[1,3];
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Abstract: Down syndrome (DS) is a common, complex disorder caused by having an extra copy of human chromosome 21 (Trisomy 21). While clinical presentation varies extensively, Alzheimer disease (AD) pathology is found in brains of virtually all people with DS by 40 years. This increases their dementia risk such that one third of the DS population develops AD by 60 years. Therefore DS allows the investigation of pathogenetic mechanisms underlying its clear genetic form of early-onset AD. To model DS in mice, a 'transchromosomic' model, Tc1, was generated carrying a freely-segregating copy of human chromosome 21 (Hsa21), which is trisomic for ~75% of Hsa21 genes. However, Tc1 is not functionally trisomic for *APP*. By crossing Tc1 with the J20 model, a transgenic mouse overexpressing mutant human *APP* that models amyloid deposition, it is possible to compare contributions of trisomy 21 and *APP* overexpression to phenotypes in the genotypically different offspring. One of the most striking phenotypes in this cross is the exacerbation of amyloid plaque deposition in mice expressing both Trisomy 21 and *APP* overexpression (Tc1;J20 mice), compared to mice overexpressing *APP* without Trisomy 21 (J20 mice). This is accompanied by a reduction in survival and increased behavioural deficits in the Tc1;J20 mice. It is therefore of interest to develop methods that would facilitate the identification of gene candidates on Hsa21 that may contribute to the aggravation of amyloid pathology in Tc1;J20 mice. To develop an *in vitro* model, which is more accessible to observation and amenable to manipulation than *in vivo* systems, we established a primary cortical culture system from early postnatal Tc1xJ20 mice. To assess the validity and utility of these cultures, they were characterized for APP expression, human A production, ratio

of neuronal to non-neuronal cells, and the proportion of mosaicism for the Tc1 chromosome. These *in vitro* phenotypes obtained were juxtaposed with relevant *in vivo* observations in Tc1xJ20 mice, allowing for the comparison of these two systems in modelling phenotypes relevant to AD. To further pinpoint AD-related phenotypes and downstream genetic pathways that may be differentially modified by genotype, we annotated and verified data obtained from a pilot RNA sequencing study of Tc1xJ20 hippocampal tissue, and identified gene candidates of interest to follow up. The work detailed here therefore discusses some approaches we have taken to identify novel genetic contributions of Hsa21, apart from *APP*, to AD phenotypes.

Disclosures: X. Choong: None. **M. Zanda:** None. **V. Plagnol:** None. **V.L.J. Tybulewicz:** None. **F.K. Wiseman:** None. **E.M.C. Fisher:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.14/C72

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Alzheimer’s disease research in the 21st century: the shift towards a new paradigm

Authors: *F. PISTOLLATO, C. P. CHANDRASEKERA;
Physicians Committee For Responsible Med., Washington, DC

Abstract: Animal models of Alzheimer disease (AD) have been extensively utilized in the last few decades in an effort to elucidate the pathophysiological mechanisms of this disease and to test novel therapeutic approaches. However, research success has not effectively translated into therapeutic success for human patients. We investigated the reasons for this translational discrepancy, presenting challenges and opportunities in AD research. Our analysis revealed that translational failure is due - at least in part - to the overuse of animal models that cannot accurately recapitulate human AD etiopathogenesis or drug responses and the inadequate use of human-based investigational methods. We propose how we can mitigate this translational barrier by employing human-based methods to elucidate disease processes occurring at multiple levels of complexity (from gene expression to protein, cellular, tissue/organ to individual and population level). Novel human-based cellular and computational models are already being applied in toxicology and regulatory testing, and the adoption and the widespread implementation of such tools in AD research will undoubtedly facilitate human-relevant data acquisition. Additionally, clinical studies focused on nutritional and lifestyle intervention strategies to reduce and/or prevent early symptoms of AD represent another relevant and

important way to elucidate AD pathogenesis and treatment options in a human-based setting. Taken together, it is clear that a paradigm shift towards human-based research is the best way to tackle the ever-increasing prevalence of AD in the 21st century.

Disclosures: **F. Pistollato:** None. **C.P. Chandrasekera:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.15/C73

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Baseline neuropathological and behavioral phenotypes for transgenic mice expressing apoE3 and apoE4

Authors: *S. HALAVI, M. DULCICH, R. E. HARTMAN;
Dept. of Psychology, Loma Linda Univ., Loma Linda, CA

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder associated with a build-up of amyloid-beta (Aβ) plaques that accumulate over time, causing neuronal dysfunction in the brain and cognitive deficits. Apolipoprotein (apoE) also plays a factor in the pathogenesis of AD, in that individuals who express apoE4 have a higher likelihood of developing AD, but apoE3 does not appear to modify the risk of developing the disease. The current study assessed whether the targeted expression of human apoE alters neuropathology in a transgenic mouse model of AD by establishing baseline neuropathological and behavioral phenotypes. All mice underwent behavioral testing (water maze, open field, rotarod, and zero maze) at 20 months old, and then brain sections were stained with HJ3.4 and thio-S to quantify Aβ. Transgenic mice expressing apoE4 had more severe spatial learning deficits than those expressing apoE3. They also spent more time in the open quadrants of the zero maze, which may be due to motor deficits and/or abnormal exploratory/risk-taking behaviors. The brains of transgenic mice expressing apoE4 stained with HJ3.4 also had more Aβ plaques in the dorsal cortex and the hippocampus combined compared to those expressing apoE3. In summary, transgenic mice expressing apoE4 had greater behavioral deficits and worse Alzheimer’s neuropathology than transgenic mice expressing apoE3.

Disclosures: **S. Halavi:** None. **M. Dulcich:** None. **R.E. Hartman:** None.

Poster

673. Alzheimer's Disease: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 673.16/C74

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: LSH framework FES0908

Title: The effect of acute BACE1 inhibition on early cognitive deficits in a mouse model of Aβ toxicity

Authors: *M. LOOS[1], E. REMMELINK[1], B. LUBBERS[1], R. E. VAN KESTEREN[2], M. VERHAGE[3], A. B. SMIT[2];
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Abstract: Alzheimer's disease (AD) is characterized by progressive neuropathological changes and decline in cognitive function. The formation of Aβ oligomers is regarded as an early event in the development of AD symptomatology. Indeed, application of Aβ oligomers affects synaptic plasticity (i.e. LTP) *in vitro* and *in vivo*. To study the consequences of Aβ oligomer-induced synaptotoxicity of on cognitive function *in vivo*, it is key to develop robust cognitive readouts that represent the direct toxic effects of oligomers before plaque deposition. However, in mouse models of AD, behavioral impairments are typically only observed at a relatively late stage, when plaque formation and other secondary pathological processes downstream of Aβ oligomerization may contribute to cognitive dysfunction. Here we describe a novel 1-night discrimination learning task to measure cognitive function in mice in an automated home-cage. In this task, that runs without any human intervention, mice could obtain all their food by passing through one of three entrances in a wall placed in front of a reward dispenser. A systemic injection of a low dose of MK-801, a non-competitive antagonist of NMDA receptors that attenuates LTP, impaired discrimination learning in wild type mice, as demonstrated by a significantly increased number of entrances to reach the learning criterion (80% through the correct entrance), pharmacologically validating this novel cognitive task. Next, we observed that transgenic mice overproducing Aβ oligomers were significantly slower at reaching the learning criterion, not only around the age at which amyloid plaques start to be visible (26 - 30 weeks of age), but also before plaque formation at 16 weeks of age. These data indicate that the synaptotoxic effects of Aβ oligomers, as previously reported *in vitro* and *in vivo*, might have directly affected discrimination learning in this task. A single acute dose of the BACE1 inhibitor LY2886721, a rate-limiting enzyme in Aβ production, was used to test the hypothesis that acute

reduction of Aβ oligomers is sufficient to restore the early cognitive deficit in the APP/PS1 mouse model.

Disclosures: **M. Loos:** A. Employment/Salary (full or part-time):; Sylics (Synaptologics BV). **E. Remmelink:** A. Employment/Salary (full or part-time):; Sylics (Synaptologics BV). **B. Lubbers:** A. Employment/Salary (full or part-time):; Sylics (Synaptologics BV). **R.E. van Kesteren:** None. **M. Verhage:** F. Consulting Fees (e.g., advisory boards); Sylics (Synaptologics BV). **A.B. Smit:** F. Consulting Fees (e.g., advisory boards); Sylics (Synaptologics BV).

Poster

674. Alzheimer's Disease: Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 674.01/C75

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1ZIANS003116-05

Title: APP-induced neurodegeneration is mediated by the unfolded protein response

Authors: *A. GUMASTE, S. JIAO, L. BELLUSCIO;
NINDS, NIH, Bethesda, MD

Abstract: The molecular mechanism underlying cell death associated with Alzheimer's disease (AD) remains largely unknown. Recent studies suggest that endoplasmic reticulum (ER) stress and the resulting Unfolded Protein Response (UPR) play an important role in neurodegeneration. The UPR is a mechanism that regulates protein translation and serves to alleviate ER stress. Protein kinase RNA-like endoplasmic Reticulum Kinase (PERK) and eukaryotic translation initiation factor 2A (eIF2a) are two key components of the UPR. We have previously shown that expression of a mutated humanized amyloid precursor protein (hAPP) in mature olfactory sensory neurons (OSN) causes extensive, visible cell death throughout the olfactory epithelium. Here, we used this AD mouse model to determine the basis of this cell loss and initially found that hAPP expression caused a significant increase in UPR-related protein levels in olfactory epithelial tissue. To test the role of PERK and eIF2a in mediating OSN loss we crossed our previously used hAPP-expressing mice into two distinct mutant lines that disrupt either PERK or eIF2a protein function. We show that suppression of either PERK or eIF2a function results in a significant reduction of UPR-protein levels, bringing them closer to wild-type levels. In addition, we found that disrupting PERK or eIF2a protein function significantly decreases caspase-3 expression, while increasing OSN survival. Overall our results suggest that intervening in UPR

activity, through suppression of PERK or eIF2a, may be a useful strategy to reduce neuronal loss associated with AD.

Disclosures: **A. Gumaste:** None. **S. Jiao:** None. **L. Belluscio:** None.

Poster

674. Alzheimer's Disease: Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 674.02/C76

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1ZIANS003116-05

Title: Perk inhibitor prevents neural death in mouse model of Alzheimer’s disease

Authors: ***S. JIAO**, A. GUMASTE, L. BELLUSCIO;
Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD

Abstract: Alzheimer’s disease (AD) is the most common form of dementia in the elderly, however, the precise molecular machinery that control the neuronal death remains unclear. Studies have shown that the unfolded protein response (UPR), a mechanism that controls initiation of protein translation, is overactive in patients with Alzheimer’s disease, which can lead to shutdown of protein synthesis and subsequent neural apoptosis. To determine whether suppressing the UPR can improve neuronal survival in an AD context we utilized an animal model, previously established in our lab that overexpress a humanized mutated amyloid precursor protein (hAPP) in olfactory sensory neurons (OSNs) and exhibits early visible apoptosis. Using these mice we applied a specific inhibitor of the Protein kinase RNA-like endoplasmic Reticulum Kinase (PERK), a critical effector of UPR pathway, via direct nasal administration and assessed changes in OSN survival. The activity of PERK and other key proteins in UPR pathway had been suppressed after just one week of nasal delivery. Interestingly, despite persistent expression of hAPP in the mutant mice, we found a significant reduction of Caspase-3 expressing OSNs and a significant increase of OMP or hAPP expressing OSNs after treatment. Examination of the olfactory bulbs further revealed that PERK inhibition enhanced OSN axonal projections and partially restored the glomerular layer structure as compared to mutant controls. Moreover, behavioral assessment using a buried food assay, clearly demonstrated that these mutant mice treated with PERK inhibitor had significantly restored their olfactory function. Together, these data suggest that PERK may be an effective therapeutic target for blocking neural loss associated with AD progression.

Disclosures: **S. Jiao:** None. **A. Gumaste:** None. **L. Belluscio:** None.

Poster

674. Alzheimer's Disease: Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 674.03/C77

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CSIR, Govt. of India, miND (BSC0115)

Title: TRB3 mediates neuronal cell death evoked by β-amyloid by a dual mechanism: apoptosis and autophagy

Authors: S. SALEEM, *S. C. BISWAS;
CSIR-Indian Inst. of Chem. Biol., Kolkata, India

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by accumulation of misfolded and aggregated proteins in combination with prolonged cellular stress. This results in an Endoplasmic Reticulum stress in an AD brain. Mounting evidence also implicates defective autophagy in the pathogenesis of AD. Autophagy is a catabolic process causing degradation of accumulated proteins. TRB3, a novel ER stress inducible gene has a probable role in neurodegeneration as implicated by recent research. In our study we find that TRB3 is upregulated in cultured cortical neurons in response to β-amyloid $(A\beta)_{1-42}$ treatment and in Aβ overexpressing transgenic mouse model. It is reported that TRB3 inhibits Akt during apoptosis. We find that TRB3 lies upstream of Akt pathway and also observe an interesting regulatory mechanism between transcription factor FoxO1, a direct target of Akt and TRB3 in Aβ-treated cells. These two proteins regulate the expression of one another in a feed forward mechanism. Our results revealed that level of FoxO1 in shTRB3 transfected cells, following Aβ treatment decreases, whereas this reduction is averted by blocking MDM2 with an inhibitor. This indicates that Akt is active in TRB3 knockdown cells, which results in MDM2 mediated degradation of FoxO1.We further observed decreased expression of Bim, a pro-apoptotic gene, upon downregulation of TRB3. Furthermore, we find that TRB3 upregulates autophagy in neurons via the Akt/mTOR pathway in Aβ-treated neurons. Downregulating TRB3 leads to increased mTOR activity. It is reported that mTOR inactivates ULK1 by phosphorylation at S-757. We find that upon TRB3 downregulation, there is sustenance of the inhibitory phosphorylation of ULK1 resulting in diminished levels of autophagy which is depicted by decreased conversion of LC3-I to LC3-II. To check the efficiency of autophagy thus induced, we monitored changes in levels of p62 upon Aβ treatment in neuronal cells. Build-up of p62 at later

time points indicates a decrease in autophagy flux in the treated cells. We therefore deduce that upregulated levels of TRB3, in neurons exposed to $(A\beta)_{1-42}$ leads to apoptotic death together with enhanced formation of autophagosomes and their accumulation in these neurons. Most importantly, we observed significant protection of primary cortical and hippocampal neurons against Aβ insult on silencing of TRB3 by shRNA. Thus, our study indicates that TRB3 may serve as a potential target for therapeutic intervention in AD.

Disclosures: S. Saleem: None. **S.C. Biswas:** None.

Poster

674. Alzheimer's Disease: Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 674.04/C78

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mechanisms of Aging and Dementia Training Grant (Northwestern University), AG20506

National Institute of Neurological Disorders and Stroke, NS085770

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National Institute on Aging (Northwestern University Alzheimer's Disease Core Center), AG13854

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The Davee Foundation Neurobiology Research Initiative Fund

Title: Histopathologic markers are related to *in vivo* cortical atrophy in primary progressive aphasia with Alzheimer's pathology

Authors: *D. T. OHM, G. KIM, A. MARTERSTECK, S. WEINTRAUB, E. BIGIO, M. MESULAM, E. ROGALSKI, C. GEULA;
Cognitive Neurol. & Alzheimer's Dis. Ctr., Northwestern Univ., Chicago, IL

Abstract: The neurobiological substrates of cortical atrophy are not well understood in neurodegenerative diseases that cause dementia. We quantified the regional specificity of

Alzheimer disease (AD) pathology (i.e., amyloid-ß plaques [APs] and neurofibrillary tangles [NFTs]) and cortical atrophy measures in two patients with primary progressive aphasia (PPA) and AD pathology (PPA-AD) that had structural magnetic resonance imaging (sMRI) within 18 months of death. PPA is a clinical dementia syndrome associated with autopsy-confirmed AD pathology in approximately 40% of cases, as well as a signature pattern of asymmetric atrophy concentrated in the left perisylvian language network. The neuroanatomical selectivity of PPA allows for unique within-subject comparisons between compromised and relatively spared regions (e.g., left versus right hemispheres; language versus memory regions). This study compared two control regions of interest (ROIs) (memory-related entorhinal cortex and primary visual cortex) to five language ROIs: 1) inferior frontal gyrus, 2) anterior superior temporal gyrus, 3) posterior superior temporal gyrus, 4) anterior inferior parietal lobule, and 5) posterior inferior parietal lobule. The cortical volume of each ROI was quantified from the PPA-AD subjects' sMRI using FreeSurfer software and compared to a group of 22 age-matched cognitively healthy adults in order to quantify volume loss. ROIs delineated by the neuroimaging analysis served as boundaries for unbiased stereology performed on whole-hemisphere sections to quantify NFT and AP densities. Both PPA-AD subjects displayed a leftward asymmetry of cortical atrophy, with the most prominent volume loss in the posterior language ROIs, especially the anterior and posterior inferior parietal lobules. Negligible volume loss was found bilaterally in control ROIs in comparison to the language ROIs. NFT densities exceeded AP densities in all ROIs except primary visual cortex for both PPA-AD subjects. The largest densities of NFTs were found within the language ROIs, while AP densities were evenly distributed across both language and control ROIs. These preliminary measures suggest that NFT deposits parallel cortical atrophy in their neuroanatomical distribution. The findings further highlight the regional selectivity of neurodegenerative markers within the perisylvian language network in PPA-AD.

Disclosures: D.T. Ohm: None. **G. Kim:** None. **A. Martersteck:** None. **S. Weintraub:** None. **E. Bigio:** None. **M. Mesulam:** None. **E. Rogalski:** None. **C. Geula:** None.

Poster

674. Alzheimer's Disease: Neurodegeneration

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SURE Grant to CC & SS

SFR Grant # 562 to DG

President's Fund for Excellence to DG

Title: Lack of evidence for peroxynitrite formation in nitric oxide-induced death in retinal cell cultures

Authors: C. COUGHLIN[1], S. SAJJAD[1], K. NELSON[1], O. ANDERSON[1], M. RAJSOMBATH[1], *D. GRAY[2];
[1]Biol., [2]Simmons Col., Boston, MA

Abstract: Alzheimer's Disease (AD) and glaucoma are two prevalent and debilitating neurodegenerative diseases that involve the beta amyloid (Ab) peptide. However, soluble Ab peptides present in healthy tissue and in cultured embryonic retinal neurons have been shown to inhibit evoked release of acetylcholine (ACh) from cultured embryonic retinal neurons. The cellular pathway of the Ab peptide involves nitric oxide (NO), guanyl cyclase (cGMP) and phosphokinase G (PKG) without inducing cell pathology or death. In late stages of these diseases, however, Ab peptide aggregation is certainly associated with cell death. One hypothesis is that NO generated by Ab activity may lead to a toxic side effect over time by combining with mitochondrial superoxides. The likeliest candidate is the toxic superoxide-NO conjugate peroxynitrite. A model of cell death was created by exposing retinal cells to high levels of NO using an NO donor (3,3-bisaminoethyl-1-hydroxy-2-oxo-1-triazene [NOC-18]) in combination with a superoxide dismutase inhibitor (1- N,N' Diethyl-dithiocarbamate, DDC). Evidence of peroxynitrite effect in this model of cell death was measured by measuring cell survival with and without a specific inhibitor of peroxynitrite (5-aminosalicylate, 5AS). There was no significant reversal of cell death in these conditions thus not supporting an endogenous role for peroxynitrite in neurodegenerative cell death. Similar results were demonstrated when Ab was used to endogenously produce NO. Future work will involve examining the concentration of NOD and DDC, incubating the cultures for a longer period of time to determine if the compounds affect the cells gradually, and using immuno-labeling to identify the specific cells in the culture.

Disclosures: C. Coughlin: None. **S. Sajjad:** None. **K. Nelson:** None. **O. Anderson:** None. **M. Rajsombath:** None. **D. Gray:** None.

Poster

674. Alzheimer's Disease: Neurodegeneration

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Topic: C.02. Alzheimer's Disease and Other Dementias

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National Science Foundation of China 91332207

Tsinghua University Initiative Scientific Program 20111080956

Title: Separation of neurodegeneration and memory loss in a *Drosophila* model of Alzheimer's disease

Authors: *Y. HU[1], B. LIANG[2], Y. ZHONG[1];
[1]Sch. of Life, Tsinghua Univ., Beijing, China; [2]HaiNan Normal Univ., HaiNan, China

Abstract: Alzheimer's disease is an age-related, non-reversible, progressive neurodegenerative disease. Memory loss and neurodegeneration are the two dramatic characteristics in Alzheimer's disease. However, lots of drug and genetic screens conducted in animal models established some candidates that can only rescue memory loss but not neurodegeneration. It indicates that there might be some factors separating memory loss and neurodegeneration. In a *Drosophila* model of Alzheimer's disease, we found that suppression of Abeta expression could rescue neuronal loss but not learning defect. Conditional low expression of Abeta is sufficient to cause learning defect. This imply that there might be some distinct mechanisms underlying Abeta-contributing memory loss and neurodegeneration in Alzheimer's disease.

Disclosures: **Y. Hu:** None. **B. Liang:** None. **Y. Zhong:** None.

Poster

674. Alzheimer's Disease: Neurodegeneration

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIA P50 AG05146

Ellison Medical Foundation AG-NS-1101-13

Title: Progressive degeneration and functional impairment of gray matter oligodendrocytes in a mouse model of Alzheimer’s disease

Authors: *E. GONZALEZ FERNANDEZ[1], J. D. ROTHSTEIN[2,3], S. KANG[1,4,2];
[1]Shriners Pediatric Hosp. Ctr., Philadelphia, PA; [2]The Solomon H. Snyder Dept. of Neurosci., [3]Dept. of Neurol., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [4]Dept. of Anat. and Cell Biol., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Although cytotoxic mechanisms of senile amyloid plaque may directly cause widespread neuronal loss in Alzheimer's disease (AD), non-neuronal cells also play active roles in disease progression. By means of patient brain imaging and examination of rodent models of AD, recent studies have revealed white matter abnormalities and related myelin pathology in the AD brain, suggesting a pathogenic role of oligodendrocytes (OLs) in this degenerative disease. In addition to myelin sheath formation, OLs mediate metabolic support for neurons by expressing the monocarboxylate transporter 1 (MCT-1), a major form of lactate transporter in the brain. Therefore, cell loss or impaired regeneration of OLs, and/or MCT-1 downregulation, will facilitate neuronal dysfunction, and will negatively impact disease outcomes. In the present study, in order to determine the homeostatic maintenance of OLs in the AD brain, we tracked age-dependent changes in mature OLs, and performed genetic fate analyses of OL progenitors (OLPs) and mature OLs in a mouse model of AD (APPswe/PSEN1-dE9). In MOBP-EGFP; APPswe/PSEN1-dE9 mice, EGFP-labeled OLs significantly decreased in an age-dependent manner, particularly in several gray matter areas, such as motor cortex and hippocampus. This cellular loss was more prominent near AB plaques. Despite the apparent OL loss, neither the number of NG2+ OLPs nor the degree of OLP proliferation was altered in the AD brain. In tamoxifen-administered (P37) PLP-CreER;ROSA26-mEGFP mice, a marked reduction of EGFP-labeled, OL process densities preceded the decrease of OL cell bodies, and such myelin abnormalities were accompanied by dystrophy of nodes of Ranvier. More interestingly, a significantly larger fraction of surviving OLs lost MCT1 expression, as assessed by tdTomato signal in OLs in APPswe/PSEN1-dE9; MOBP-EGFP; MCT1-tdTomato mice. Finally, the fate analysis of OLPs (from P35) in PDGFaR-CreER;ROSA26-mEGFP mice did not show any sign of compensatory increases in new OL differentiation in AD. Rather, it appeared that the number of newly born OLs was decreased in parallel to the gradual OL loss. Taken together, these results suggest that OL impairments are relatively early events in the progression of AD, and that OL dysfunctions are multifaceted, involving demyelination, cell death, failure of metabolic support, as well as the failure of their own regeneration.

Disclosures: E. Gonzalez Fernandez: None. **J.D. Rothstein:** None. **S. Kang:** None.

Poster

674. Alzheimer's Disease: Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 674.08/C82

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Acceleration Partners

Rosenberg Alzheimer's Project

Title: Targeting the stress pathway in cognitive decline

Authors: *P. SPILMAN[1], J. CAMPAGNA[1], B. JAGODZINSKA[1], K. POKSAY[2], O. GOROSTIZA[2], A. MATALIS[2], D. E. BREDESEN[1,2], V. JOHN[1];
[1]Neurol., Easton Ctr. for Alzheimer's Dis. Research, Univ. of California, Los Angeles, CA;
[2]Bredesen Lab., Buck Inst. for Res. on Aging, Novato, CA

Abstract: Background. Stress is associated with cognitive decline [Tschanz *Int Rev Psychiatry* 2013], and increases risk for Alzheimer's disease (AD). Corticotropin-releasing factor receptor (CRF1) stimulation increases the phosphorylation of tau (p-tau) [Rissman *PNAS* 2012] leading to formation of neurofibrillary tangles [Carroll *J. Neurosci*. 2011], a hallmark of AD. We tested CRF1 receptor antagonists *in vitro* and *in vivo* to determine effects on p-tau levels. We posit that drug therapy alone will not be sufficient to treat cognitive decline, therefore an individualized multi-modal lifestyle intervention program - Metabolic Enhancement for Neurodegeneration (MEND) - was developed and utilized in the treatment of patients reporting cognitive decline. Stress is one of the key factors addressed in the MEND program [Bredesen, *Aging* 2014]. **Methods**. CRF-1 antagonists were tested in SH-SY5Y cells at 1 uM with/without 100 nM CRF; the small molecule "J03" was found to decrease p-tau as determined by AlphaLISA (Perkin-Elmer). It then underwent efficacy testing *in vivo* in an AD mouse model at 10 mkd by subcutaneous injection for 14-days in comparison to vehicle-only. To increase cortisol signaling, isolation stress was induced by single-housing during treatment. Cognition as reflected by working object memory was assessed pre- and end-study using Novel Object Recognition. Both tau and p-tau were determined in brain tissue using the Perkin-Elmer AlphaLISA. In the MEND pilot study, ten patients reporting memory loss confirmed by cognitive assessment were enrolled. Nine had Mild Cognitive Impairment (MCI) and one advanced AD. These patients were required to participate in stress-reducing activities such as exercise, meditation, yoga, and music with allowances for personal choice. Other factors contributing to stress such as sleep disturbances were also addressed. **Results.** The CRF-1 inhibitor J03 decreased p-tau both *in vitro* and *in vivo* in brain tissue and cognitive improvements were tightly correlated to p-tau levels. After participation in MEND for a minimum of 9 months, nine of ten patients were improved; most returned to work. Only the moderate to late stage AD patient did not improve significantly. **Conclusions.** The J03-induced improvement in cognition further supports a connection between stress, p-tau and cognition. Future studies include synthesis and testing of analogs and study of the biochemical pathways affected. The small pilot MEND study suggests a larger clinical trial is

warranted. Improved cognition in patients complaining of memory deficits by stress reduction indicates alleviating chronic stress may be an important element in effective treatment.

Disclosures: **P. Spilman:** None. **J. Campagna:** None. **B. Jagodzinska:** None. **K. Poksay:** None. **O. Gorostiza:** None. **A. Matalis:** None. **D.E. Bredesen:** None. **V. John:** None.

Poster

674. Alzheimer's Disease: Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 674.09/C83

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grants R181741110

NIH R21AG039596

Alzheimer's association

Title: Presenilin 1-induced apoptosis is mainly mediated γ-secretase-independently by PSAP and also γ-secretase-dependently by FLIP

Authors: L. ZENG[1], F. ZHANG[1], C. HU[1], D. C. XU[2], M.-Z. CUI[1], M.-Z. CUI[1], *X. XU[3]; [1]Univ. of Tennessee, Knoxville, TN; [2]Princeton Univ., Princeton, NJ; [3]Pathobiology, Univ. Tennessee, Knoxville, TN

Abstract: Presenilin 1 (PS1) has been implicated in apoptosis; however, its mechanism remains elusive. We report that PS1-induced apoptosis was associated with c-FLIP turnover and that γ-secretase inhibitors blocked c-FLIP turnover and also partially blocked PS1-induced apoptosis. A complete inhibition of PS1-induced apoptosis was achieved by knockdown of PS1-associated protein (PSAP), a mitochondrial proapoptotic protein that forms a complex with Bax upon induction of apoptosis, in the presence of γ-secretase inhibitors. PS1-induced apoptosis was partially inhibited by knockdown of caspase-8, FADD, or Bid. However, knockdown of Bax or overexpression of Bcl-2 resulted in complete inhibition of PS1-induced apoptosis. These data suggest that PS1 induces apoptosis through two pathways: the γ-secretase-dependent pathway mediated by turnover of c-FLIP and the γ-secretase-independent pathway mediated by PSAP-Bax complex formation. These two pathways converge on Bax to activate mitochondria-dependent apoptosis. These findings provide new insight into the mechanisms by which PS1 is involved in apoptosis and the mechanism by which PS1 exerts its pathogenic effects. In addition, our results strongly suggest that c-FLIP is a new substrate of γ-secretase.

Disclosures: **L. Zeng:** None. **F. Zhang:** None. **C. Hu:** None. **D.C. Xu:** None. **M. Cui:** None. **M. Cui:** None. **X. Xu:** None.

Poster

674. Alzheimer's Disease: Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 674.10/C84

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG029401.

DOVE Fellowship

Title: Progressive degeneration of monoaminergic afferents in the J20 line of amyloid precursor protein mouse model

Authors: ***C. GALLARDO**[1], S. E. LESNE[2], M. K. LEE[2];
[1]Univ. of Minnesota - Twin Cities, Minneapolis, MN; [2]Neurosci., Univ. of Minnesota, University of Minnesota - Twin Cities, MN

Abstract: Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and cause of dementia afflicting over 5 million individuals in the United States. Histopathologically, AD is characterized by the presence of extracellular senile plaques (SPs), intraneuronal neurofibrillary tangles(NFTs)/neuropil threads(NTs) and overt neurodegeneration. Previously, we showed that the progressive degeneration of subcortical monoaminergic (MAergic) neurons seen in human AD is recapitulated in the APPswe/PS1ΔE9 (APP/PS1) transgenic mouse model (Liu et al.,J Neurosci 28:13805, 2008). In this study, we set out to determine if the amyloid-dependent, progressive MAergic neurodegeneration is a general feature of animal models of cerebral amyloid pathology by examining the integrity of MAergic neurons in the J20 mouse model. We show that the MAergic afferents are significantly lost with the aging and amyloid accumulation in the transgenic J20 line. Consistent with the prominent hippocampal amyloid pathology, greater loss of hippocampal MAergic afferents were observed. Our results indicate that Aβ pathology is sufficient to induce progressive neurodegeneration in multiple, independent transgenic lines of amyloid deposition. Furthermore, assessing the integrity of brainstem populations in AD mouse models provides a feasible model to study AD-related neurodegeneration *in vivo*.

Disclosures: **C. Gallardo:** None. **S.E. Lesne:** None. **M.K. Lee:** None.

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.01/C85

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U01 AG024904

R01 EB008281

R01 EB008432

Title: Alzheimer's disease classification with novel microstructural metrics from diffusion-weighted MRI

Authors: *T. M. NIR[1], J. E. VILLALON-REINA[1], B. GUTMAN[1], N. JAHANSHAD[1], L. ZHAN[1], C. R. JACK, Jr[2], M. W. WEINER[3], P. M. THOMPSON[1];
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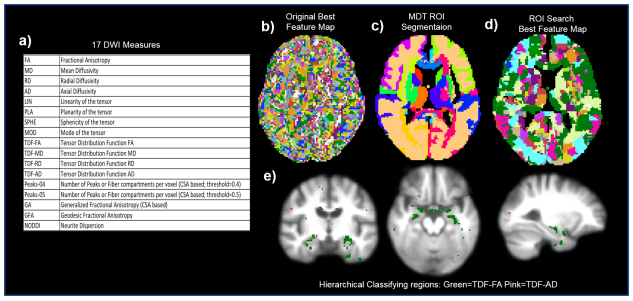
Abstract: Alzheimer's disease (AD) deficits may be due in part to declining white matter (WM) integrity and disrupted connectivity, detectable using diffusion weighted MRI (dMRI). New microstructural measures derived from various dMRI models may carry different information about WM microstructure including the level of diffusion anisotropy, diffusivity, complexity, number of fiber compartments, number of crossing fibers and neurite dispersion. Here we aimed to find the most helpful dMRI metrics and brain regions to classify people into diagnostic group (AD vs CN), from a set of 17 dMRI-derived feature maps. Structural T1-weighted MRI and dMRI images (5 b0/41 diffusion-weighted) were collected from 53 CN (72.3+/-6.2 y; 24M/27F), and 47 AD patients (73.7+/-8.0 y; 27M/20F) as part of the ADNI initiative. After correcting for eddy current and EPI distortions, we computed 17 DWI microstructural measures (Fig 1a) from 4 different reconstruction models: diffusion tensor (DTI), orientation distribution function (CSA-ODF), tensor distribution function (TDF), and neurite orientation dispersion (NODDI). All maps were elastically normalized to a minimal deformation template (MDT). The MDT was segmented into cortical, subcortical and WM ROIs (Fig 1c). For each voxel, 17 separate 10-fold cross-validated logistic regression classifiers were run per DWI measure. The DWI measure with lowest mean squared error was selected for each voxel (Fig 1b). Individual voxels are noisy and neighboring voxels are not independent, so the best measure for each voxel was changed to the measure most frequently found in its neighborhood, constrained by its ROI (Fig 1d). A second

logistic regression classifier with an elastic net regularizer (a mixture of L1 and L2 penalties) was run on the resulting feature map (cross validating for tuning parameters). Classification accuracy was 82%. Voxels contributing to the classification were found in the hippocampus and temporal lobe (Fig 1e). The best metrics in these voxels were TDF-FA and TDF-AD - suggesting non-tensor diffusion models may be useful for classification.



Disclosures: **T.M. Nir:** None. **J.E. Villalon-Reina:** None. **B. Gutman:** None. **N. Jahanshad:** None. **L. Zhan:** None. **C.R. Jack:** None. **M.W. Weiner:** None. **P.M. Thompson:** None.

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

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The Wyncote Foundation

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Title: Cognitive reserve is differentially associated with grey matter atrophy in frontotemporal lobar degeneration and Alzheimer’s disease

Authors: *K. PLACEK, L. MASSIMO, C. OLM, D. IRWIN, V. M. Y. LEE, J. Q. TROJANOWSKI, V. M. VAN DEERLIN, C. T. MCMILLAN, M. GROSSMAN;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Frontotemporal lobar degeneration (FTLD) and Alzheimer's disease (AD) are neurodegenerative conditions characterized by a progressive decline in neuroanatomical structure and cognitive function. However, there is increasing evidence for heterogeneous rates of decline across individuals, and the mechanisms of this heterogeneity are poorly understood. One theory suggests that cognitive reserve (CR) can provide a protective benefit on the clinical manifestation of neurodegenerative disease. According to this account, individuals with higher intellectual achievement are able to sustain cognitive function despite significant neuronal injury. Here, we investigated the relationship between CR and brain atrophy as assessed by grey matter (GM) in AD and FTLD. GM density was retrospectively calculated from T1-weighted MRI in AD (N=98) and FTLD (N=120) patients with pathology confirmed by autopsy, a known pathogenic mutation, or a cross-validated cerebrospinal fluid ratio of total-tau to amyloid-beta1-42. To assess CR, we created a composite cognitive reserve index (CRI) comprised of ordinal measures of lifetime educational and occupational attainment. In FTLD patients, higher CRI scores were associated with greater GM in frontal and temporal regions known to be affected by FTLD pathology. Conversely, in AD patients, higher CRI scores were associated with lesser GM in parietal, temporal, and occipital regions known to be affected by AD pathology. These effects held after accounting for age and disease duration. Our findings suggest that CR is differentially associated with GM atrophy in FTLD and AD. These findings are consistent with a previous report suggesting that CR is associated with longer survival in FTLD and reduced survival in AD. We suggest that the protective benefit on GM conferred by CR in FTLD likely reflects an earlier clinical presentation due to increased sensitivity to symptom detection, given the locus of disease in frontotemporal regions important to executive functioning. Given the primarily temporoparietal locus of AD, AD patients with higher CR likely require more brain atrophy in affected regions to exhibit cognitive decline and thus exhibit decreased GM compared with lower-CR patients due to delayed clinical presentation. Together, these findings suggest a differential mechanism of CR in AD and FTLD-related neurodegenerative disease.

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Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.03/C87

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Quantitative EEG characterization of mAChR1: the use of qEEG as a potential biomarker for therapeutic targets of Alzheimer's disease

Authors: S. GARSON, A. GOTTER, J. STEVENS, S. FOX, P. L. TANNENBAUM, A. SAVITZ, L. S. LUBBERS, M. H. PAUSCH, D. C. BESHORE, J. M. USLANER, C. J. WINROW, *Z. WU;
Neurol., Merck & Co., West Point, PA

Abstract: Muscarinic acetylcholine receptor 1 (mAChR1) are localized in forebrain areas including cerebral cortex, hippocampus, and striatum and are involved in mediating higher cognitive processes, such as learning and memory, making it an attractive therapeutic target for Alzheimer's disease (AD). A selective mAChR1 positive allosteric modulator (PAM), PQCA, has been shown to improve cognitive performance in various rodents and non-human primate models relevant to AD. Electroencephalography (EEG) and quantitative electroencephalography (qEEG) are important tools in quantifying changes in brain activity. Neurological diseases such as AD often affect the relative power of qEEG signals resulting in a "slowing" of the qEEG signal which corresponds to an increase of low-frequency spectral power bands and a decrease of higher-frequency bands. qEEG studies were conducted in ambulatory animals via radio telemetry to characterize the effects of the acetylcholinesterase inhibitor donepezil, the nonselective mAChR antagonist scopolamine, the nonselective mAChR agonist Xanomeline, and the M1-selective PAM PQCA. Telemetric physiological monitors were implanted subcutaneously, and included electrodes for EEG and EMG recording. Effects of each compound were analyzed and compared to within-animal vehicle controls. A custom-developed qEEG analysis algorithm was coded and compiled in Matlab to perform artifact rejection and short-time Fourier transform on EEG signals. qEEG data were analyzed from 1 to 100 Hz in 1 Hz increments in 3-second intervals and banded in standard frequency ranges (Delta, Theta, Alpha, Sigma, Beta, Gamma) and subsequently averaged into 30-minute time bins across 24-hours. Scopolamine induced increases in low to mid frequency powers (Delta 0.5Hz -Beta 19.0-30Hz) and decreases in high frequency gamma power (35.0-100.0 Hz), a similar pattern to that observed in AD patients. On the other hand, activating muscarinic receptors through acetylcholinesterase inhibitor, muscarinic receptor agonist or M1 selective PAM produced an opposite pattern of effects by reducing the qEEG signal in low to mid frequency powers and increasing high frequency gamma power. Additionally, Xanomeline, Donepezil and PQCA all increased the time spent in wake with concurrent reductions in NREM and REM sleep. These results support that qEEG could be an effective biomarker for the evaluation of potential mAChR-based AD treatments.

Disclosures: **S. Garson:** A. Employment/Salary (full or part-time):; Merck & Co. **A. Gotter:** A. Employment/Salary (full or part-time):; Merck & Co. **J. Stevens:** A. Employment/Salary (full

or part-time):; Merck & Co. **S. Fox:** A. Employment/Salary (full or part-time):; Merck & Co. **P.L. Tannenbaum:** A. Employment/Salary (full or part-time):; Merck & Co. **A. Savitz:** A. Employment/Salary (full or part-time):; Merck & Co. **L.S. Lubbers:** A. Employment/Salary (full or part-time):; Merck & Co. **M.H. Pausch:** A. Employment/Salary (full or part-time):; Merck & Co. **D.C. Beshore:** A. Employment/Salary (full or part-time):; Merck & Co. **J.M. Uslaner:** A. Employment/Salary (full or part-time):; Merck & Co. **C.J. Winrow:** A. Employment/Salary (full or part-time):; Merck & Co. **Z. Wu:** A. Employment/Salary (full or part-time):; Merck & Co..

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

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Program#/Poster#: 675.04/C88

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P30 AG028383

NIH Grant P01 AG030128

NIH Grant R01 AG033036

Title: White matter microstructure in an empirically-derived fornix template is associated with neuropathological markers of Alzheimer’s disease

Authors: *C. BROWN[1], G. A. JICHA[2], F. A. SCHMITT[2], L. J. VAN ELDIK[3], C. D. SMITH[4], B. T. GOLD[5];
[1]Anat. and Neurobio., [2]Neurology, Sanders-Brown Ctr. on Aging, [3]Sanders-Brown Ctr. on Aging, [4]Neurol., [5]Anat. and Neurobiology, Sanders-Brown Ctr. on Aging, Univ. of Kentucky, Lexington, KY

Abstract: Alzheimer’s disease (AD) has a prolonged preclinical stage, during which pathological proteins accumulate and alterations to brain structure and function begin to appear. Recent evidence suggests that, along with amyloid deposition, decline in white matter microstructure in the fornix may be an early preclinical process. The fornix is the primary white matter tract responsible for hippocampal output, and declines in white matter microstructure in this tract appear to precede hippocampal atrophy. However, widely available atlases of the fornix include only subsections of the tract, typically in the form of multiple submasks, limiting the ability and simplicity of assessing white matter microstructure in the fornix as a continuous

structure. In the present study, a new fornix template was developed using probabilistic tractography in adults ranging from 25-77 years old and then applied to an independent cohort of cognitively normal older adults (ages 65-92) to investigate the relationship between white matter microstructure and cerebrospinal fluid (CSF) biomarkers of preclinical AD. Ninety-five adults ranging from 25-77 years old underwent diffusion tensor imaging, which was used to perform probabilistic tractography. Tractography used hippocampal seeds and waypoint masks based on the current JHU-ICBM White Matter Labels Atlas to develop a continuous fornix from the fimbria through the body. This fornix template was applied to a separate cohort of 34 cognitively normal older adults for whom CSF levels of β-amyloid ($A\beta_{42}$), total tau, and phosphorylated tau ($p\text{-}tau_{181}$) were available. Correlation analyses revealed that fractional anisotropy (FA) in the fornix template was positively correlated with CSF $A\beta_{42}$ ($r = 0.46$, $p = 0.008$), negatively correlated with CSF $p\text{-}tau_{181}/A\beta_{42}$ ratio ($r = -0.38$, $p = .03$), and marginally negatively correlated with CSF total tau ($r = -0.30$, $p = 0.09$) when controlling for age and sex. In contrast, FA in the current (JHU-ICBM) labels atlas had only marginal positive correlation with CSF $A\beta_{42}$ levels ($r = 0.32$, $p = 0.07$) and had no correlation with other CSF markers ($p > 0.22$) when controlling for age and sex. Importantly, a global FA measure had no relationship with any CSF marker ($p > 0.14$). These findings indicate that there is a relationship between lower white matter microstructure in the fornix and early markers of Alzheimer's disease, particularly increasing $A\beta_{42}$ burden. The new fornix template provides a valid and sensitive tool for measuring age-related alterations to white matter microstructure. The new fornix mask may also provide an additional neuroimaging biomarker of preclinical AD pathology for use in future research.

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Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.05/C89

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

NSERC

FQRS

Weston Brain Institute

Alzheimer's Society

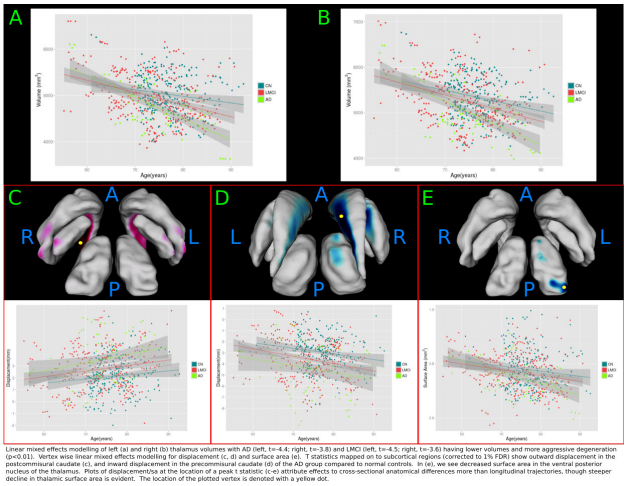
Brain Canada

Michael J. Fox Foundation for Parkinson's Research

Title: Subcortical volume and morphology in Alzheimer's disease and mild cognitive impairment

Authors: *M. R. PATEL[1], G. DEVENYI[1], V. KONG[1,2], M. CHAKRAVARTY[1,3]; [1]Cerebral Imaging Ctr., Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [2]Integrated Program in Neurosci., [3]Departments of Psychiatry and Biomed. Engin., McGill Univ., Montreal, QC, Canada

Abstract: Background The neurodegenerative processes in Alzheimer's disease have been well-characterized at the level of the neocortex and the hippocampus. Surprisingly, subcortical structures such as striatum, thalamus, and pallidum have received little attention. Here, we analyze these structures a longitudinal fashion. Methods 800 T1-weighted images from ADNI1 (291/509 1.5T/3T) were preprocessed with minc-bpipe-library then processed using MAGeT brain, which outputs volume, vertex displacement (DP), and vertex surface area (SA) on a per structure basis. 602 passed a manual quality control (79/291/232 AD/MCI/NC, 328/274 F/M). Effects of diagnosis were examined using a linear mixed effects model, accounting for age, and sex. Modelling of volume changes was done with and without covarying for ICV to explore correlations of diagnosis and volume. DP and SA were corrected for multiple comparisons with FDR. Results We observed decreased bilateral thalamic volume ($p<0.01$) in Alzheimer's patients (AD) (left, t=-4.4; right, t=-3.8) and late mild cognitive impairment patients (LMCI) (left, t=-4.5; right, t=-3.6) populations compared to normal controls (NC). AD and LMCI patients had increased inward DP in the anterior thalamus and decreased SA in the ventral posterior nucleus of the thalamus. Compared to NC, we found bilateral increased inward DP in the precommissural caudate, counterbalanced by increased outward DP in the postcommissural caudate in AD and LMCI patients (1% FDR). In the pallidum, we found bilateral inward DP at the anterior end and outward DP at the posterior end (1% FDR). No significant differences were found between AD and MCI groups. Conclusion We found that the thalamus shows pronounced degeneration in AD and LMCI patients at a steeper trajectory. We saw that AD and LMCI caudate show a posterior bulging out DP, suggesting an accompanying thinning of the nearby internal capsule. Finally, we found that subcortical structures do not appear to be major factors in distinguishing AD from MCI patients, but may be involved in the onset of the prodromal of Alzheimer's disease.



Linear mixed effects modelling of left (a) and right (b) thalamus volumes with AD (left, t=-4.4; right, t=-3.8) and LMCI (left, t=-4.5; right, t=-3.6) having lower volumes and more aggressive degeneration (p<0.01). Vertex wise linear mixed effects modelling for displacement (c, d) and surface area (e). T statistics mapped on to subcortical regions (corrected to 1% FDR) show outward displacement in the posterior commissural caudate (c), and inward displacement in the precommissural caudate (d) of the AD group compared to normal controls. In (e), we see decreased surface area in the ventral posterior nucleus of the thalamus. Plots of displacement/sa at the location of a peak t statistic (*) attribute effects to cross-sectional anatomical differences more than longitudinal trajectories, though steeper decline in thalamic surface area is evident. The location of the plotted vertex is denoted with a yellow dot.

Disclosures: **M.R. Patel:** None. **G. Devenyi:** None. **V. Kong:** None. **M. Chakravarty:** None.

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.06/C90

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Brain Canada

Alzheimer's Society

Canadian Institutes of Health Research (CIHR)

Title: Heterogeneity in neuroanatomical differences in relation to amyloid burden in mild cognitive impairment

Authors: ***V. KONG**[1,2], G. DEVENYI[1], R. PATEL[1], M. CHAKRAVARTY[1,3];
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Abstract: Introduction Amyloid deposits have long been considered to be related to the initiation of Alzheimer’s disease (AD), but a high amyloid burden alone does not predict the diagnosis of AD. The goal of the project was to determine neuroanatomical differences between subjects with high and low amyloid burden and to investigate how those differ in the diagnostic groups of AD, mild cognitive impairment (MCI) and cognitively normal controls (NL). Methods

3458 3T T1-weighted magnetic resonance images (MRI) and analyzed florbetapir (FBP) positron emission tomography data were obtained from the Alzheimer's Disease Neuroimaging Initiative database for 860 subjects (141 AD, 456 MCI, 263 NL; baseline to 3 years). Subjects were divided into high and low amyloid groups, using average FBP standardized uptake rate with cerebellum as reference. MRI scans were processed using the CIVET pipeline for cortical thickness (CT), MAGeT Brain algorithm and BEaST for hippocampal volume (HV) and morphometry using surface displacement (HD). Linear mixed-effects model is used to examine the effect of amyloid burden on CT, HV and HD for all subjects (covaried for age, gender, baseline diagnosis and total brain volume) and within each diagnostic group (covaried for age, gender and total brain volume). Results There was significant reduction for high amyloid subjects in both left and right CT and HV and a further significant interaction between high amyloid burden and MCI. When stratified into diagnostic groups, NL and AD subjects showed no significant differences in all the measures, while MCI subjects have significant decrease in both left and right CT in the medial temporal and posterior association cortex regions. No hippocampal shape differences were observed. Discussion & Conclusion Here we show that high amyloid in MCI subjects is related to accelerated neurodegeneration in HV and CT, while differences are not pronounced in NL and AD subjects. NL subjects with high amyloid are able to maintain integrity, while AD subjects, regardless of amyloid burden, had considerable atrophy to show any difference in anatomy.

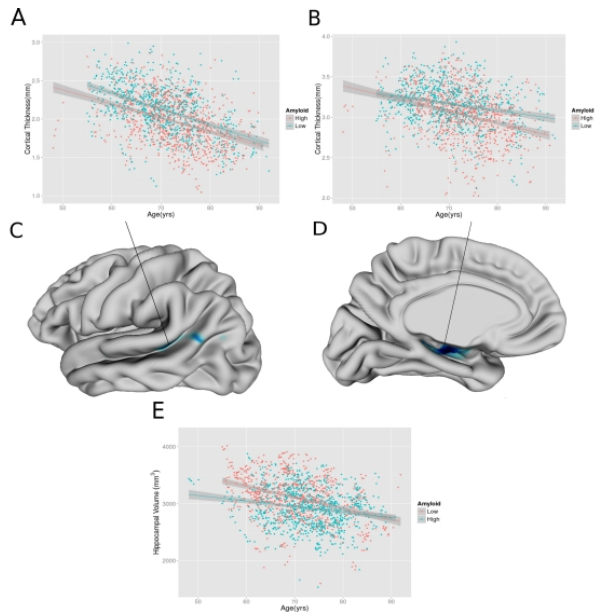

Figure. Differences of left hemisphere CT and HV in MCI subjects. C,D) CT difference between subjects high and low amyloid after FDR correction of 5%. A,B) CT differences over age at vertices selected from C and D. E) HV for subjects with high and low amyloid over age with linear regression lines

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Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.07/C91

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: 5-HT2c agonists-induced changes in cerebral blood oxygen-level dependent (BOLD) pharmacologic magnetic resonance imaging (phMRI) in awake rats and blockade with 5-HT2c antagonist: a potential pharmacodynamic biomarker

Authors: ***S. J. BAKER**[1], G. FOX[1], K. DRESCHER[2], J. BEAVER[1], A. M. BASSO[1]; [1]ZR13, AbbVie Labs, North Chicago, IL; [2]GmbH & Co.KG, AbbVie Labs, Ludwigshafen, Germany

Abstract: 5-HT_{2C} receptor activation plays a critical role modulating neurotransmission and brain circuits relevant to the pathophysiology of neuropsychiatric disorders, drug addiction and obesity. Preclinical studies and clinical trials with 5-HT_{2C} receptor agonists (Lorcaserin, Vabicaserin) indicate that 5-HT_{2C} biology translates from preclinical species to humans. The Vabicaserin clinical trials, however, provide no receptor occupancy or translational biomarker data. 5-HT_{2C} receptor occupancy and pharmacodynamic effects on brain function across rodents, NHPs and healthy humans could be used for dose selection in early clinical studies. At this time, however, no selective 5-HT_{2C} PET tracer is available to interrogate receptor occupancy and the plasma concentration relationship for 5-HT_{2C} receptor agonists. phMRI is an established imaging modality that can measure changes in BOLD activity following administration of pharmacological agents with regional specificity. The goal of the present study was to evaluate phMRI as a pharmacodynamic/*in vivo* biomarker for functional dose-response effects of 5-HT_{2C} agonists and the receptor specificity of this response. Previous phMRI studies by others demonstrated that mCPP, a 5-HT_{2C} receptor agonist, induces comparable effects on BOLD signal in anesthetized rats and healthy humans. Male Sprague-Dawley rats (350-450g) were used for phMRI studies. Following a mock MRI training regimen, animals were scanned using a restrainer specifically designed for small-animal awake MRI on a 70/30 Bruker Biospec: eight-shot spin-echo EPI sequence, TR/TE = 3200/50ms, in-plane resolution= 250 μm^2 in a 128 x 128 2D voxel matrix, slice thickness = 1.25 mm and inter-slice spacing = 1.5 mm. Total scan time for a single experiment was 42 min (10 minute baseline-2minute i.p. infusion-30 minute post-dose). BOLD signal percent-change was interpreted using VivoQuant (Invicro; Boston, MA) software. Average maps were created using analysis of functional neuro-images (AFNI) (Cox et al., 2005). The current study confirms the findings using mCPP in awake rats and expanded the dataset to include another 5-HT_{2C} agonist compound, Vabicaserin. Vabicaserin (1-30 mg/kg) induced dose-dependent increases in BOLD signal in brain regions consistent with the distribution of 5-HT_{2C} receptors and neuronal projections. SB-242084 (1 mg/kg) attenuated the increases in BOLD signal induced by mCPP, and Vabicaserin in conscious rats, suggesting that BOLD changes are

specifically mediated by 5-HT_{2C} receptors. Further studies are planned to investigate whether the BOLD signal changes observed in rodents will translate to a higher species, NHPs.

Disclosures: **S.J. Baker:** A. Employment/Salary (full or part-time):; AbbVie Labs full-time employee. **G. Fox:** A. Employment/Salary (full or part-time):; AbbVie Labs full-time employee. **K. Drescher:** A. Employment/Salary (full or part-time):; AbbVie Labs full-time employee. **J. Beaver:** A. Employment/Salary (full or part-time):; AbbVie Labs full-time employee. **A.M. Basso:** A. Employment/Salary (full or part-time):; AbbVie Labs full-time employee.

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH STTR grant R42-NS055475

Title: Rapid, fully automated method for quantitative analysis of PET amyloid scans in Alzheimer’s disease

Authors: ***P. H. KUO**[1,2], P. K. BHARADWAJ[3], W. P. KRAFFT[1], M. C. FITZHUGH[3], G. E. ALEXANDER[3,4,5,6,7], G. ZUBAL[8];
[1]Med. Imaging, [2]Biomed. Engin., Univ. of Arizona Col. of Med., Tucson, AZ; [3]Psychology, [4]Neurosci. Grad. Interdisciplinary Program, [5]Physiological Sci. Grad. Interdisciplinary Program, [6]Evelyn F. McKnight Brain Inst., Univ. of Arizona, Tucson, AZ; [7]Arizona Alzheimer's Consortium, Phoenix, AZ; [8]Z-Concepts LLC, East Haven, CT

Abstract: Amyloid imaging by positron emission tomography (PET) plays a critical role in patient selection for clinical trials for Alzheimer’s disease (AD) and is also poised for more routine clinical use. Unlike analyses of CSF for beta amyloid or tau, PET amyloid imaging provides information on the regional distribution of amyloid in brain. While visual read is the clinically approved method for interpretation of amyloid imaging, software quantification is vital for research and may also become an effective tool for computer-aided diagnosis. Important features of quantification software include diagnostic accuracy, reproducibility, speed, and resource requirements. The Alzheimer’s Disease Neuroimaging Initiative (ADNI) has collected a cohort of controls and AD patients’ clinical information, neuroimaging scans, and biomarkers. The ADNI database provides quantitative analyses of amyloid imaging with the application of a semi-automated method using Freesurfer (FS) software that can typically require hours to

analyze a scan. In contrast, the Alzheimer's Disease Evaluation of Radiotracers (ADER) software is a fully automated program that yields quantitative, reproducible regional results of amyloid deposition and can analyze a scan in less than a minute using a standard laptop computer. We report initial results using ADNI data to compare ADER and a semi-automated method with FS using discriminant analysis to determine classification accuracy between patients with AD and controls. In the ADNI database, a semi-automated processing stream involving registration of PET images with structural scans and cortical parcellation with FS is used to obtain a mean SUVr from four regions. The average value from a similar set of four regions for ADER (frontal, parietal, anterior cingulate, and precuneus) was then used for our evaluation. Only those ADER processed scans with a good quality control rating were used, including 93 controls and 64 AD patients. Discriminant analysis using the mean of 4 regions calculated by ADER produced an overall classification accuracy of 80%, controls' classification accuracy of 88% and AD classification of 67%. Discriminant analysis using the mean of 4 regions calculated by the semi-automated FS method resulted in an overall classification accuracy of 82%, controls' classification accuracy of 89% and AD classification of 72%. A linear regression analysis comparing the two methods showed good agreement (adjusted R2 = 0.71). These preliminary results show that the fully automated ADER achieves advantages of speed and lower resource requirements without sacrificing overall diagnostic accuracy compared to a semi-automated method with FS.

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Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.09/C93

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 7R01NS034467

Title: Apolipoprotein E4 allele differentially modulates cerebral blood flow and blood-brain barrier permeability in Alzheimer's disease

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Abstract: Apolipoprotein E (APOE) has three isoforms: APOE2, APOE3, and APOE4. APOE4 is a major genetic risk factor for Alzheimer's disease (AD), a debilitating dementia characterized by early and progressive neurovascular dysfunction. APOE4 has direct effects on the cerebrovascular system, resulting in microvascular lesions and blood-brain barrier (BBB) damage. Using high-resolution dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) to simultaneously map BBB integrity regionally and quantitatively, our recent study in humans demonstrated BBB disruption restricted to the hippocampus in aging and in patients with mild cognitive impairment (MCI). In this new MRI study, we first analyzed disruptions in brain structure and function in humans with genetic risk of AD (APOE4 carriers vs. non-carriers) who were cognitively normal or MCI. We show accelerated BBB breakdown in APOE4 carriers, particularly in the hippocampal region. BBB breakdown is also highly correlated with loss in white matter integrity, measured using diffusion tensor imaging (DTI)-derived fractional anisotropy and mean diffusivity. Furthermore, we find reduced cerebral blood flow (CBF) using arterial spin labeling (ASL) method in the hippocampus and subcortical regions in MCI individuals, which worsens in APOE4 carriers. We conducted similar experiments in transgenic mice carrying 5 familial-AD mutations (5xFAD) that overexpress human amyloid-beta (Aβ), and express human APOE3 or APOE4 (E3/E4FAD). We show an age-dependent increased BBB permeability in the cortex and hippocampus of wildtype animals, consistent with our recent findings in humans. Notably, we provide the first *in vivo* preclinical evidence revealing that E4FAD mice have an increased cortical/hippocampal BBB permeability compared to age-matched E3FAD mice, which correlates with the magnitude of BBB breakdown using *in vivo* two-photon imaging. Correlation between DTI metrics and hippocampal BBB permeability measures are also found in AD mice. Finally, we show a decrease in CBF in the cortex and hippocampus of E4FAD mice compared to age-matched E3FAD mice using a dynamic susceptibility contrast (DSC) method. These new MRI findings corroborate our recent study in humans showing that individuals carrying the E4 allele may be predisposed to accelerated pericyte loss and greater BBB damage. In sum, we provide evidence in both humans and mice illustrating accelerated neurovascular alterations in APOE4 carriers, emphasizing that brain microcirculation and BBB integrity drive the initial pathological changes in the living brain leading to cognitive decline and neuronal loss.

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Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

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Program#/Poster#: 675.10/C94

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The design, study conduct, and financial support for this research was provided by AbbVie.

Title: Electrophysiological alterations by 5-HT2C receptor agonist CP-809,101 in sleep EEG and power spectral activity

Authors: P. VESELCIC, Y. MORDASHOVA, *K. M. WICKE;
Abbvie Germany Gmbh & Co KG, Ludwigshafen, Germany

Abstract: The serotonergic 5-HT2C receptor is a key contributor to a variety of medical conditions including psychiatric and neurological diseases. Therapeutic approaches at this receptor, with both, agonists and antagonists continue to emerge1. To better understand 5-HT2C mediated function we have investigated electrophysiological changes in sleep structure and EEG power spectral distribution by the highly selective 5-HT2C receptor agonist CP-809,101. In two independent studies, male Fischer rats with chronically implanted supracortical EEG-electrodes were treated with 10 mg/kg of CP-809,101. In the 1st study, sleep structure changes in terms of total sleep time, percent of time spent in different sleep stages, the number of rapid eye movement (REM) episodes, and latency to first sleep and REM episode were analyzed. Treatment with CP-809,101 led to attenuation of time spent in mild, deep, and REM sleep. It increased time spent awake and latency to first sleep and REM episode. The 2nd study investigated power spectral distribution changes revealing an attenuation of delta and theta band by CP-809,101 in comparison to vehicle and baseline recordings while maintaining the delta/theta ratio. A refined statistical method for analysis of power spectral changes was applied. Our results clearly demonstrate that acute treatment with CP-809,101 changes sleep and power spectral parameters in a mutual way. 5-HT2C agonists have been suggested to exhibit antidepressant-like profile that fits to the sleep changes observed in our study. Further, 5-HT2C

agonists have been reported to inhibit theta oscillation, desynchronizing the EEG and leading to shifts to lower frequencies2,3. Yet, despite the inhibition of theta oscillation and desynchronization of the EEG by CP-809,101, the ratio between delta/theta revealed no changes underlying the wake-promoting effects of CP-809,101. 1 Rosenzweig-Lipson et al 2011 Neuropsychopharmacol. 36:363 2 Hajos et al 2003 JPET 306:605 3 Sörman et al 2011 Neuropharmacol 61:489 Disclosures: All authors are employees of AbbVie. The design, study conduct, and financial support for this research was provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Disclosures: **P. Veselcic:** A. Employment/Salary (full or part-time):; AbbVie Germany GmbH & Co KG. **Y. Mordashova:** A. Employment/Salary (full or part-time):; AbbVie Germany GmbH & Co KG. **K.M. Wicke:** A. Employment/Salary (full or part-time):; AbbVie Germany GmbH & Co KG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie Germany GmbH & Co KG.

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.11/C95

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Synchrotron x-ray fluorescence indicates enhanced zinc load in plaques of CRND8 animals supplemented with dietary copper

Authors: *P. A. KAKALEC[1], K. N. BOGGS[2], S. N. HOWELL[2], C. M. GROEBER TRAVIS[3], J. M. FLINN[1];
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Abstract: Amyloid beta (Aβ) plaques characteristic of Alzheimer’s disease contain varying levels of trace metals such as copper, iron, zinc, and aluminum, which may influence and accelerate their aggregation. Metals such as iron have been reported in tissue surrounding plaques, while copper and zinc have been shown to directly bind to Aβ. Furthermore, zinc being the second most abundant trace metal in the body, is required for many physiological processes such as protein folding and enzyme function. In fact, APP itself actually contains a zinc binding site in its promoter region, and its expression is tightly regulated by zinc-containing transcription factors (Vostrov & Quitschke, 1997). There is considerable information available to the general

population, which supports dietary enrichment of trace metals and minerals through supplementation. As much as half of the U.S. population takes daily vitamins enhanced with copper, iron, zinc, and other minerals, with the idea that there is no such thing as too much supplementation (Radimer et al., 2004). This is an alarming misconception, as researchers have shown that alterations in homeostatic levels of these trace metals not only cause abnormalities in plaque load, but they can also cause or exacerbate memory impairment. To examine the effect of dietary metal supplementation on metal content in Alzheimer's plaques, CRND8 animals (n=16) were administered enhanced drinking water for 5 months: Fe (10ppm FeNO3), Fe+Cu (10ppm FeNO3 + 0.2ppm CuNO3), Cu (0.2ppm CuNO3), and lab tap water. Water was analyzed regularly using inductively coupled plasma-optical emission spectroscopy and ion chromatography at the United States Geological Survey (USGS, Reston, VA) to confirm metal content. Congo red staining was first performed in these animals in order to locate and confirm plaques. Upon confirmation of plaques, slides were taken to National Synchrotron Light Source at Brookhaven National Laboratory for analysis of both bound and free metal content. With the use of beamline X-26A total zinc load was measured at levels below 100 ppm in each of the four water conditions. Statistical analysis of zinc content showed significantly higher levels of zinc in CRND8 animals supplemented with copper water ($p= 0.014$) compared to iron water. Additional analyses are ongoing.

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Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.12/C96

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Concurrence of mild cognitive impairment and increased cerebral iron load, as measured by quantitative susceptibility mapping, is associated with increased frontotemporal functional connectivity at rest

Authors: *J. VAN BERGEN[1,2], X. LI[2], M. WYSS[3], J. HUA[2], S. SCHREINER[1], S. STEININGER[1], F. QUEVENCO[1], S. LEH[1], A. GIETL[1], R. NITSCH[1], K. PRUESSMANN[3], P. C. M. VAN ZIJL[2], C. HOCK[1], P. G. UNSCHULD[1];
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Kirby center for Functional Brain Imaging, Kennedy Krieger Inst. and Johns Hopkins Sch. of Med., Baltimore, MD; [3]Inst. for Biomed. Engin., ETH Zurich, Zurich, Switzerland

Abstract: Introduction Altered functional connectivity during preclinical Alzheimer's Disease (AD) has been shown to be a robust finding by several resting state fMRI (rs-fMRI) studies. Various cognitive networks are implicated in brain change associated with AD-pathology and alterations of the default mode network (DMN) are considered to particularly reflect compensatory mechanisms for neuronal damage. On the other hand increases in cerebral iron may reflect pathological alteration in a context of neurodegeneration. Developments in the field of Quantitative Susceptibility Mapping (QSM) have made it possible to directly map brain tissue magnetic susceptibility, which has been shown to correlate well with tissue iron concentration in most brain gray matters. This study aims to assess a potential functional signature of iron accumulation during the stage of Mild Cognitive Impairment (MCI). **Methods** Eighteen subjects with MCI (11 male, 7 female; age 75.0 ± 7.2) and twenty-two cognitively normal elderly controls (14 male, 8 female; age 72.0 ± 5.3 were scanned on a 7T Philips Achieva System. All participants received psychiatric examination and were categorized as cognitively normal or MCI. rs-fMRI was acquired using a 3D T2-prep GRE sequence (TR = 2s, TR_{GRE}/TE_{GRE} = 3.08/1.6ms, 1.5x1.5x1.5 mm^3). Phase data for susceptibility measurements was acquired using a multi-echo 3D GRE scans (TR/TE/ΔTE=23/6/6ms, 0.5x0.5x0.5mm^3). Susceptibility maps were reconstructed by sequentially applying Laplacian based phase unwrapping, sophisticated harmonic artifact reduction for phase data (SHARP) and LSQR based minimization. Classification in “high” and “low” cerebral iron content was performed by a median split of the average cortical grey matter susceptibility in frontal, parietal, temporal and occipital lobes. The iron classification and MCI status were used as the covariates in connectivity analysis using the CONN MATLAB toolbox. **Results** Strong (p-FDR-corrected < 0.001, $T_{1,23}$=4.32) increase of activity in a frontotemperal region for the combined effect of MCI status and “high” iron load was observed. Prominent structures in these region include Frontal Pole and Gyrus, Temporal Pole and Gyrus, Cingulate Gyrus, Paracingulate Gyrus, Amygdala and Nucleus Accumbens. **Discussion and Conclusion** Our data demonstrate increased frontotemporal connectivity in a context of high cerebral iron, as indicated by QSM, and MCI. Further research on the relationship between susceptibility and pathological correlates of AD, such as Amyloid beta plaque density, appears promising for investigation of physiological correlates of altered functional connectivity during preclinical AD.

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Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

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Program#/Poster#: 675.13/D1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG037639

R01 AG027161

P50 AG033514

Title: Family history of Alzheimer's disease is associated with myelin content in preclinical Alzheimer's disease

Authors: *C.-M. A. CANDA[1], J. SOJKOVA[2,6], D. C. DEAN, III[7], J. P. O'GRADY[3], S. HURLEY[8], N. J. DAVENPORT[3,4], O. C. OKONKWO[3,6], S. ASTHANA[3,6,4], M. A. SAGER[3,4], S. C. JOHNSON[3,6,4], A. L. ALEXANDER[5], B. B. BENDLIN[3,6];
[1]Alzheimer's Dis. Res. Ctr., Univ. of Wisconsin Sch. of Med. and Pub, Madison, WI; [3]Alzheimer's Dis. Res. Ctr., [4]Wisconsin Alzheimer's Inst., [5]Waisman Ctr., [2]Univ. of Wisconsin Sch. of Med. and Publ. Hlth., Madison, WI; [6]Geriatric Res. Educ. and Clin. Ctr., Wm. S. Middleton Mem. VA Hosp., Madison, WI; [7]Waisman Ctr., Univ. of Wisconsin Madison, Madison, WI; [8]Oxford Ctr. for Functional Magnetic Resonance Imaging of the Brain, Univ. of Oxford, Oxford, United Kingdom

Abstract: Background: Parental family history (FH) of Alzheimer's disease is associated with greater risk for developing the disease, as well as several preclinical brain changes. Previous work from our laboratory has found an effect of maternal family history on cerebral white matter, but it is unknown if the effect is due to axonal or myelin degeneration. The objective of the current study was to assess the effect of FH on myelin, using novel Multi-component Driven Equilibrium Single-Pulse Observation of T1 and T2 (mcDESPOT) imaging, which is sensitive to myelin content. We hypothesized that FH, particularly maternal FH would have a negative effect on myelin. **Methods:** 128 participants (mean age: 61.72 ± 6.32 years; range 45-74 years) from the Wisconsin Registry for Alzheimer's Prevention underwent mcDESPOT on a GE MR750 3T to calculate myelin water fraction (MWF) maps. Parental FH of AD was based on probable or confirmed AD of one or both parents determined through structured interview or determined through autopsy and reviewed by a multidisciplinary diagnostic consensus panel. Absence of parental FH required that the participant's father survive to at least age 70 and the mother to age 75 without incurring a formal diagnosis of dementia or exhibiting cognitive deterioration. A voxel-wise two-sample t-test in SPM12 was used to compare positive family history and

negative family history, in addition to specifically examining the effect of maternal family history on MWF, adjusting for age and sex. **Results:** Positive parental family history was associated with higher MWF. Affected regions were localized primarily to frontal, parietal, and occipital cortical regions. Participants with maternal family history specifically, showed higher WMF compared to participants without FH, in addition to higher WMF compared to participants with paternal FH. **Conclusions:** Prior studies suggest that parental FH of AD is associated with brain differences, even in asymptomatic individuals. This is the first study to suggest myelin differences based on parental FH. Contrary to our hypothesis, we found evidence for higher myelin levels among individuals with parental FH, particularly maternal family history. Given the strong risk for AD associated with maternal FH, further study is needed to determine the mechanism which underlies increased myelin content among participants who are vulnerable to AD.

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Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

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Program#/Poster#: 675.14/D2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5R01AG013308

NIH Grant F31AG047041

Title: Genetic risk scores for Alzheimer’s disease are associated with thinning of hippocampal complex subregions

Authors: *T. M. HARRISON[1], E. P. LAU[2], Z. MAHMOOD[2], A. C. BURGGREN[2], G. W. SMALL[2], S. Y. BOOKHEIMER[2];
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Abstract: Twenty-one genetic loci have been identified for which specific variants increase an individual’s risk for sporadic, late-onset Alzheimer Disease (AD). Many of these risk genes have been identified using genome-wide association studies. An important unresolved question is whether or not polygenic risk scores that use combinations of risk loci increase power to detect

changes in neuroimaging biomarkers for AD. Further, the optimal method to calculate polygenic risk scores is an active field of research. In a pilot study, we acquired high-resolution structural images of the hippocampus in 47 healthy, older subjects. For 15 of these subjects, longitudinal two-year follow-up data were also available. Unweighted and weighted polygenic risk scores for AD were calculated for each subject. The unweighted risk score (URS) was the sum of family history of AD (0 if negative history or 1 if positive history), APOE4 alleles (0,1, or 2), CLU risk alleles (0,1, or 2) and PICALM risk alleles (0,1, or 2). The weighted risk scores (WRS) used published odds ratios (OR) to weight the relative contribution of these risk factors before summing: positive family history OR=2, APOE4 OR=3, CLU minor allele OR=0.9, PICALM minor allele OR=0.9. For the cross-sectional cohort, both URS and WRS showed no relationship to thickness in any hippocampal subregion. For the longitudinal cohort, URS and WRS correlated strongly to percent change in thickness across the whole hippocampus (URS r=-0.85, p=0.0001; WRS r=-0.63, p=0.015), driven by strong relationships in the entorhinal cortex (URS r=-0.66, p=0.01; WRS r=-0.73, p=0.003) and CA23/dentate gyrus (URS r=-0.66, p=0.01; WRS r=-0.65, p=0.01), two anterior subregions of the hippocampal complex. In a multiple regression including age and sex as predictors, models with URS (beta=-2.16, p=0.0003) and WRS (beta=-7.01, p=0.014) predicting percent change in thickness across the whole hippocampus were significant (URS model p=0.009; WRS model p=0.03). These results provide compelling evidence that polygenic AD-risk scores may be especially sensitive to structural change over time in regions affected early in AD, like the hippocampus. Our findings also show that the relationships between our polygenic risk score and hippocampal thinning are not mediated by weighting risk score components with published ORs.

Disclosures: T.M. Harrison: None. **E.P. Lau:** None. **Z. Mahmood:** None. **A.C. Burggren:** None. **G.W. Small:** None. **S.Y. Bookheimer:** None.

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.15/D3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH-NIA: P50-AG033514

Title: Intracranial 4D blood flow analyses in the Alzheimer’s disease spectrum

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Abstract: INTRODUCTION: Although viewed as separate clinical entities, the line between Alzheimer's disease (AD) and vascular dementia is becoming increasingly blurred, and research on the vascular contributions to AD is of utmost importance as they may exacerbate AD pathology and clinical symptoms. 4D Flow MRI with phase contrast vastly undersampled isotropic projection imaging (pcVIPR) allows researchers to obtain comprehensive measurements of intracranial vascular health, such as mean flow in the arteries of the Circle of Willis. It remains to be determined if and how pcVIPR metrics correlate with perfusion-weighted imaging using Arterial Spin Labeling (ASL). Evidence indicates that cerebral blood flow (CBF) is lower in AD, and hypoperfusion is also present in similar regions in individuals with mild cognitive impairment and/or with genetic risk for AD. However, the relationship between perfusion weighted CBF and metrics of cerebral artery flow and health has not been clearly established *in vivo* across the spectrum of AD severity. METHODS: 130 middle aged adults, 42 cognitively healthy older adults, 28 MCI patients, and 18 AD patients (N = 218, mean age 63.2, SD: 9.8) underwent 4D Flow MRI using pcVIPR as well as ASL imaging with a 1525 tagging delay. Statistical parametric mapping version 12 (SPM12) software was used for voxel-wise regression analysis, with middle cerebral artery (MCA) mean flow as the predictor variable, age and gender as covariates, and CBF measured via ASL as the outcome variable. RESULTS: Average bilateral MCA mean flow was positively associated with ASL whole brain perfusion (max t = 7.61) in frontoparietal regions, including the precuneus, a brain region known to be involved in the pathogenesis of AD. All significant clusters survived familywise error (FWE) correction with p values < 0.05 using a critical t-value of 4.24. CONCLUSION: With any novel technology, it is important to determine how it may inform or add to existing methods. ASL is a commonly used technique in AD research, and this study helps to link intracranial arterial flow measured via pcVIPR to local cerebral perfusion delivered to capillary beds measured in ASL. These data demonstrate a consistent pattern of disrupted blood flow, using two different techniques, across the AD spectrum. Further characterization of ASL and pcVIPR metrics are needed to clarify the relationship between vascular health and AD.

Disclosures: **S.E. Berman:** None. **L. Rivera:** None. **L.R. Clark:** None. **A.M. Racine:** None. **C. Illingworth:** None. **J.M. Oh:** None. **P. Cary:** A. Employment/Salary (full or part-time):; University of Wisconsin-Madison. **C.M. Carlsson:** None. **B.B. Bendlin:** None. **S. Asthana:** None. **P. Turski:** None. **H. Rowley:** None. **O. Wieben:** None. **S.C. Johnson:** None.

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.16/D4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MINZ Grant

Title: Local shape differences are associated with amyloid beta load and neuropsychological performance in cognitively normal elderly individuals

Authors: *C. SCHROEDER[1], A. GIETL[1], M. M. CHAKRAVARTY[2], M. M. PARK[2], L. MICHELS[3], S. KOLLIAS[3], P. G. UNSCHULD[1], S. L. KROLL[1], A. M. KÄLIN[1], C. HOCK[1], S. E. LEH-SEAL[1];
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Abstract: Amyloid plaque and tangle formation is a hallmark of Alzheimer disease (AD). Pittsburgh compound B positron emission tomography (PiB-PET) measures amyloid beta (Aβ) load, which increases substantially before clinical symptoms appear in AD. Aβ load has been shown to be associated with risk of conversion to very mild AD in cognitively normal elderly individuals. Changes in amyloid PET are thought to precede volumetric changes assessed by structural (T1) magnetic resonance imaging (MRI) such as hippocampal atrophy. Because of the vast and growing number of at-risk individuals in our society, a biomarker derived from T1 data that becomes abnormal comparably early to amyloid PET would be highly valuable given the ready availability and non-invasive nature of MRI. Our sample comprised 69 cognitively normal elderly subjects (age 55-80 years, 32 female). Our measures included standardized uptake value ratio (SUVR) from dynamic PiB-PET imaging, volumes of numerous brain regions from 3 Tesla MRI, cortical thickness (CT), shapes of subcortical structures, and performance in neuropsychological tests. Segmentation of subcortical structures was performed using the MAGeT Brain algorithm. We tested associations between those measures controlling for the influence of age. We found bilateral negative associations between SUVR and CT in the entorhinal cortex (EC) ($p = 0.05$, FDR-corrected) and within these regions, EC CT predicted performance in a test of executive function (EF). Left-hemispheric associations between SUVR and shape were present in the globus pallidus (GP) and thalamus ($p = 0.1$, FDR-corrected): In the GP, associations were present in the posterior medial nucleus for outward and in the anterior lateral nucleus for inward displacements. Within this region of the medial nucleus, inward displacements predicted performance in a test of EF. In the thalamus, associations were present

in the anterior, medial, and lateral dorsal nucleus for inward and in the pulvinar and ventral posterior nucleus for outward displacements. The EC is affected early by AD pathology and CT in this region was bilaterally associated with SUVR in healthy elderly individuals. Shapes of subcortical structures showed left-hemispheric associations with SUVR, in line with findings of stronger left- versus right-hemispheric abnormalities in mild AD. Shape in the left medial pallidal nucleus, which is involved in EF, was associated with SUVR and performance in a test of EF, as was CT of bilateral EC. These findings suggest that local shape differences parallel behaviorally relevant pathological states at the presymptomatic stage of neurodegenerative diseases.

Disclosures: C. Schroeder: None. **A. Gietl:** None. **M.M. Chakravarty:** None. **M.M. Park:** None. **L. Michels:** None. **S. Kollias:** None. **P.G. Unschuld:** None. **S.L. Kroll:** None. **A.M. Kälin:** None. **C. Hock:** None. **S.E. Leh-Seal:** None.

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.17/D5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH BD2k

NIH U01 AG024904

Title: Comparison of diffusion weighted imaging protocols for investigating Alzheimer’s disease in ADNI

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Abstract: Diffusion weighted imaging (DWI) is a popular neuroimaging tool in clinical research to identify biomarkers for neurodegenerative diseases. In preparation for its third phase and to allow improved processing and tractography methods, the Alzheimer’s Disease Neuroimaging Initiative (ADNI) has begun acquiring higher directional resolution enhanced DWIs (eDWI; 68 diffusion-weighted and 8 b0 images) in addition to its original DWI scanning protocol (oDWI; 46 diffusion-weighted and 5 b0 images) during the same MRI session. While the protocol

changes are considered improvements, it is crucial to characterize any differences across scan protocols to maintain empirical integrity. We used identical processing methods for both the eDWI and oDWI scans according to the ENIGMA-DTI protocol, which is designed to generate robust measures across DWI acquisition schemes. We assessed the sensitivity to protocol changes of diffusion tensor imaging (DTI)-derived scalar measures of white matter microstructure in 34 subjects from the ADNI dataset (age: 74±6.8; M/F: 22/13; 12 CN, 12 eMCI, 8 lMCI, 2 AD). Fractional anisotropy (FA) and mean diffusivity (MD) measures were extracted from the white matter skeleton and averaged across regions of interest (ROIs). With a paired t-test, we compared the enhanced and original DTI data at each ROI. 7 of the 63 ROIs studied yielded significantly different measurements of FA, and 49 of 63 were significantly different for MD (after controlling for the multiple tests with the false discovery rate procedure; $q = 0.05$; Table 1). FA measurements may be less sensitive to the protocol change than MD measurements as the DWI's angular resolution increases. Average FA ROIs were generally higher in the oDWI than the eDWI. In this small sample, no significant differences were detected between controls and those with lMCI and AD when testing each protocol individually. These preliminary results suggest the need to further assess differences between the two types of images with a larger data set. More enhanced DWI scans are currently being acquired as part of ADNI and should make for an insightful comparison of protocols.

Table 1. Average ROI values of significant measures

ROI	average MD oDWI	average MD eDWI	ROI	average FA oDWI	average FA eDWI
SLF-L	8x10-4	7x10-4	CGH-R	0.484	0.461
FX	2.1x10-3	1x10-3	UNC-L	0.465	0.448
ACR-L	8x10-4	8x10-4	CST	0.611	0.583
SCR-L	8x10-4	7x10-4	CST-L	0.616	0.589
CR-L	8x10-4	8x10-4	CST-R	0.605	0.577
ACR	8x10-4	8x10-4	FX	0.348	0.363
SLF	8x10-4	7x10-4	CGH	0.479	0.488
ACR-R	8x10-4	8x10-4			
SCR	8x10-4	7x10-4			
CR	8x10-4	8x10-4			

PCR-L	8x10-4	8x10-4			
SCR-R	SCR-R	7x10-4			
CR-R	8x10-4	8x10-4			
SLF-R	8x10-4	7x10-4			
SS-L	8x10-4	8x10-4			
SFO-L	8x10-4	7x10-4			
SFO	8x10-4	7x10-4			
UNC-L	1x10-3	1x10-3			
RLIC-L	8x10-4	8x10-4			

Disclosures: **A. Zavaliangos-Petropulu:** None. **N. Jahanshad:** None. **C. Jack:** None. **M. Weiner:** None. **M.A. Bernstein:** None. **R.I. Reid:** None. **P.M. Thompson:** None.

Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.18/D6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Structurally distinct amyloid-β species among Alzheimer’s disease patients - revisiting a Pittsburgh compound B-refractory case

Authors: *J. MAHLER[1,2], J. RASMUSSEN[1,2], M. I. DIAMOND[3], K. P. R. NILSSON[4], L. C. WALKER[5], F. BAUMANN[1,2], M. JUCKER[1,2];
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Abstract: The abnormal aggregation of amyloidogenic proteins characterizes many neurodegenerative diseases and underlies the formation of the canonical pathological features of Alzheimer's disease (AD). Recent findings suggest that the amyloid-β (Aβ) peptide, which constitutes one of the principal aggregating proteins in AD, can adopt distinct structural conformations, reminiscent of prion strains. Indeed, in transgenic mouse models, strain-like variations of Aβ lesions can be seeded and propagated. However, the origin of these diverse structural characteristics and their relation to the clinical phenotype in AD patients remains obscure. With the aim of further analyzing variant molecular conformations among aggregated Aβ species, we used novel, conformation-sensitive methods to probe the molecular architecture of multimeric Aβ in an unusual case of AD that showed abnormally reduced high-affinity binding of Pittsburgh compound B (PIB). Specialized fluorescent dyes (luminescent conjugated oligothiophenes) as well as a novel flow cytometry assay, both designed to discriminate different amyloid structures, reliably distinguished between the PIB-refractory case and PIB-positive AD cases. These results provide evidence for the structural diversity of Aβ conformers among AD patients, which may be at the root of individual disease phenotypes.

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Poster

675. Alzheimer's Disease: Clinical Detection and Biomarkers

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 675.19/D7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Differences in inmunosignal distribution pattern for Aβ and pTau between late-onset sporadic and early-onset familial Alzheimer's disease

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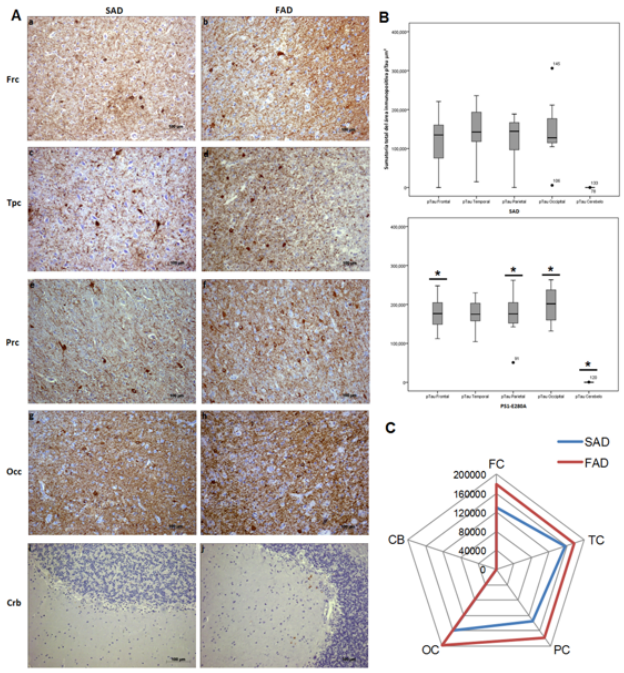
Abstract: Neuropathologically, early-onset familial Alzheimer's disease (EOFAD) shows severe Aβ pathology, pronounced brain atrophy and a distinct hyperphosphorylated tau-related pathology that extends to cerebellar cells. In this case series study, a comparative neuropathologycal analysis was made from one group of EOFAD patients and one group with late-onset sporadic Alzheimer's disease (SAD). Deparaffinized slides from the cerebral cortex and cerebellum of EOFAD and SAD patients were obtained from the Brain Bank of the

Neurosciences group of the Universidad de Antioquia. This region in northwestern Colombia, currently comprises the largest population of EOFAD subjects that carries the E280A mutation in PS1. It was observed that EOFAD cases present larger deposits in frontal, temporal, parietal and cerebellar areas; together with higher Aβ immunosignal in the cerebellum. Additionally, SAD cases present smaller Aβ deposits, but in greater quantity, in the temporal and occipital cortex compared with EOFAD. The morphometric analysis of the total immunoreactive burden and Aβ deposits also show that in general, both familial and sporadic patients have a higher immunoreactive Aβ burden in the frontal cortex when compared to the other regions analyzed. Compared with sporadic Alzheimer's disease, EOFAD has some distinctive features. The accumulation of amyloid peptide in familial patients is higher than in sporadic patients matched during CERAD and Braak staging, especially in the form of diffuse plaques. Regarding neurofibril pathology quantification, EOFAD presents itself with higher levels of hyperphosphorylated Tau (pTau) in frontal, parietal, occipital and cerebellar areas. In conclusion, there are neuropathological differences between EOFAD and SAD that could be related with the pathophysiological mechanisms of aggregation and distribution of abnormal deposits in the hereditary variant of Alzheimer's disease. These findings could have further implications on clinicopathological correlations and may also have differential therapeutic impact on those patients.



Disclosures: **D.M. Trujillo:** None. **H. Arboleda:** None. **F. Lopera:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.01/D8

Topic: C.03. Parkinson's Disease

Support: Faculty of Medicine

Title: Behavioural and pet-ct striatal evaluation after the unilateral implantation of dopamine in striatum achieves to attenuate motor abnormalities in hemiParkinsonian rat model

Authors: *P. VERGARA-ARAGON[1], M. VALVERDE AGUILAR[2], M. PALOMERO RIVAS[3], M. VELAZQUEZ PANIAGUA[4], I. SÁNCHEZ CERVANTES[4], I. LOPEZ MARTINEZ[5], L. COLIN BARENQUE[5], R. MAYEN DIAZ[4], D. VÁZQUEZ MATÍAS[4], K. PINEDA ROMERO[4], R. GONZALEZ TREJO[4], A. SOLANA ROJAS[4], P. VERGARA ARAGÓN[4];
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Abstract: The aim of this study was to evaluate the effect produced by a dopamine releasing implant in striatum in a hemiparkinsonism rat model on motor impairment, 11C-Dihydrotetrabenazine (11[C]DTBZ) uptake by Positron Emission Tomography (PET) to determine the correlation between the DTBZ to VMAT2 binding levels in the striatum, due is widely considered to be a stable marker of dopamine neurone integrity. Methods: 50 male Wistar rats divided in three groups: A) 8 control rats, B) 12 injured rats by administration of the dopaminergic specific neurotoxin 6-OHDA (8µg/4µl) by stereoscopic surgery in left median forebrain bundle (Lx), C) 20 rats with the same lesion and the colocation of a dopamine continuous release titanium dioxide implant (Lx+TiO2DA) and D) 10 rats only with the colocation of implant (TiO2DA). 21 days after the implantation all the groups were underwent to apomorphine spin-induced test (SIT) to determine damage in dopaminergic system. PET scans were performed on left and right striatum measuring the 11[C]-DHTZ uptake. Brains were obtained and mashed to analyze the dopamine content in Striatum by HPLC. Data were analyzed by one-way ANOVA; p values < 0.001 were considered significant. Results: SIT showed an increase of spins in injured animals compared to control and implanted rats: Lx vs control: 289.1 ± 26.32 vs 3.778 ± 2.682, Lx vs Lx+TiO2DA: 289.1 ±26.32 vs 29.89 ±12.33, Lx vs TiO2DA: 289.1 ±26.32 vs 13.67± 7.890. Nevertheless Lx+TiO2DA had an increased vs control: 29.89 12.33 vs 3.778 ± 2.682. PET scans disclosed on the left striatum an increase of uptake in the injured animals compared to implanted animals: Control vs Lx: 4403±1204 vs 8767 ±1422, Lx vs Lx+TiO2DA: 8767±1422 vs 1842±604.6, Lx vs TiO2DA: 8767±1422 vs 3348 ±1427. There is no difference between Control vs Lx+TiO2DA, TiO2DA and Lx+TiO2DA vs TiO2DA. The

right striatum displayed the same pattern: Control vs Lx: 4248±1157 vs 8577±1213, Lx vs Lx+TiO2DA: 1957± 683.1 vs 8577±1213 Lx vs TiO2DA: 3396 ±1547 vs 8577±1213. Here also is no any evident difference between Control vs Lx+TiO2DA, TiO2DA and Lx+TiO2DA vs TiO2DA. HPLC analysis disclosed that striatal dopamine in control animals was: 170.1 ±292.9 ng; in Lx rats: 24.66± 27.34ng, Lx+TiO2DA animals: 29.15± 43.35ng; and TiO2DA rats:123.9± 116.6ng. Conclusion: We conclude that the application of the TiO2DA implant after 21 days could improve the motor function evidenced by the spin-induced test, and this improvement is also evident with the diminishment of 11[C]DTBZ uptake by the implanted animals. In both cases this improvement was not total, but significant.

Disclosures: **P. Vergara-Aragon:** None. **M. Valverde Aguilar:** None. **M. Palomero Rivas:** None. **M. Velazquez Paniagua:** None. **I. Sánchez Cervantes:** None. **I. Lopez Martinez:** None. **L. Colin Barenque:** None. **R. Mayen Diaz:** None. **D. Vázquez Matías:** None. **K. Pineda Romero:** None. **R. Gonzalez Trejo:** None. **A. Solana Rojas:** None. **P. Vergara Aragón:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.02/D9

Topic: C.03. Parkinson’s Disease

Support: Academy of Finland

Title: Stable and highly reproducible low dose 6-OHDA model of Parkinson’s disease

Authors: ***A.-M. PENTTINEN**[1], J. ANTTILA[1], K. ALBERT[1], M. H. VOUTILAINEN[1], R. K. TUOMINEN[2], M. AIRAVAARA[1];
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Abstract: 6-OHDA (6-hydroxydopamine) animal model is widely used in the drug development for Parkinson's disease. Intrastriatal administration of 6-OHDA induces immediate degeneration of the dopaminergic nerve terminals in close proximity of the injection site accompanied by more slowly progressing retrograde cell loss in substantia nigra. Therefore it provides a therapeutic window suitable for neuroprotection and neurorestoration studies. We compared eight different experimental 6-OHDA injection set-ups to find the stable and highly reproducible low dose partial 6-OHDA model of PD. We tested seven different doses from 3 µg to 28 µg of 6-OHDA, three microinjection needles and different striatal injection sites in order to find injection

paradigm better suited for neuroprotection or neurorestoration studies and to reduce the number of used laboratory animals. The progress of lesions was evaluated by amphetamine-induced rotations and the severity of the lesions was assessed by tyrosine hydroxylase (TH) and dopamine transporter (DAT) immunohistochemistry. According to our results, a stable and highly reproducible lesion model can be induced with low doses of 6-OHDA distributed evenly into multiple injection sites (3 x 1 μg , 3 x 2 μg or 3 x 3 μg). Loss of striatal TH- and DAT positive neurites induced by these low doses is at the same level as the loss induced by higher doses (2 x 10 μg and 3 x 7 μg). The stability and reproducibility of the lesion can be enhanced by using a microinjection needle with small outer diameter and small injection volume. Furthermore, injecting the 6-OHDA in 10 degree angle can improve the anatomical targeting into striatum. Together all these factors account for the stability and reproducibility of the lesion and further decrease the number of used animals in the experiments.

Disclosures: **A. Penttinen:** None. **J. Anttila:** None. **K. Albert:** None. **M.H. Voutilainen:** None. **R.K. Tuominen:** None. **M. Airavaara:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.03/D10

Topic: C.03. Parkinson’s Disease

Support: Swedish Research Council

European Community FP7

Title: Frontocortical overexpression of alpha-synuclein in adult rats reproduces executive cognitive deficits related to Parkinson´s disease

Authors: H. S. LINDGREN[1], D. S. TAIT[3], V. FRANCARDO[1], Z. BIMPISIDIS[1], M. LUNDBLAD[2], V. J. BROWN[3], S. B. DUNNETT[4], *M. A. CENCI[1];
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Abstract: Cognitive and psychiatric symptoms are a major cause of disability in Parkinson´s Disease (PD) and do not respond to current treatments. These features are likely to depend on an

interaction between cortical alpha (α)-synuclein-related pathology, aminergic deficits, and neurovascular abnormalities. The relative contribution of different cortical regions and pathogenic factors is poorly understood. In this study, we set out to develop a rat model of frontocortical α-synuclein overexpression and related cognitive deficits that would facilitate future pathophysiological investigations. Methods: Rats received injections of adeno-associated viral (AAV) vectors coding for human wild-type α-synuclein into the medial prefrontal cortex. Transgene expression was driven by a strong neuron-specific promoter (synapsin-1). Rats injected with a control vector (AAV-GFP) and rats with an ibotenic acid lesion of the same prefrontal region served as controls. Eight weeks post AAV-injection, rats were tested in an attentional set-shifting task and in an operant delayed matching-to-position task. In parallel, animals were used to study the dynamics of potassium-evoked glutamate release in the striatum. Results: AAV- α-syn injections resulted in strong expression of human α-syn in neuronal cell bodies within the medial prefrontal cortex, and in axons within the target regions of prefrontocortical projections. Compared to AAV-GFP-injected rats, α-synuclein overexpressing animals were impaired in the ability to shift attentional sets and to acquire a delayed matching-to-position task. *In vivo* amperometry revealed attenuated potassium-evoked glutamate release, and altered kinetics of both glutamate uptake and release in the striatum. Similar though more pronounced behavioural-neurochemical findings were obtained in rats injected with ibotenic acid into the same prefrontal region. Conclusion: Frontocortical overexpression of α-synuclein causes executive cognitive deficits and pathological features relevant to PD. This model may therefore have face validity for translational research on mechanisms and treatment approaches to cognitive dysfunction in PD.

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Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.04/D11

Topic: C.03. Parkinson’s Disease

Support: Department of Pharmacology/Federal University of Santa Catarina

CNPq

CAPES

FAPESC

FINEP

Title: Effects of melatonin on non-motor symptoms and oxidative stress induced by a single intranasal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice, an animal model of Parkinson's disease

Authors: *J. M. MACK[1], T. M. MOURA[1], C. L. GONÇALVES[2], A. L. DAFRE[2], M. FARINA[2], R. D. S. PREDIGER[1];
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Abstract: Parkinson's disease (PD) is characterized by motor and non-motor symptoms that are related with the progressive degeneration of dopaminergic neurons in the nigrostriatal pathway. Our research group previously demonstrated that a single intranasal (i.n.) administration of the pro-neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is able to induce olfactory, emotional, cognitive and motor impairments in rodents associated with time-dependent loss of dopaminergic neurons in different brain areas. The aim of the present study was to evaluate the potential of the repeated administration of exogenous melatonin, a neurohormone produced and released by the pineal gland that has been associated with neuroprotective effects in PD, to prevent behavioral and neurochemical changes induced by a single i.n. MPTP administration in mice. All procedures were approved by local Ethical Committee in Animal Research (PP0830/2012). Male C57BL/6 mice (6 months-old) received melatonin (30 mg/kg) or vehicle (saline) by intraperitoneal route during 25 consecutive days. On the 5th day of treatment animals were anaesthetized with isoflurane (0.96%) and subject to a single i.n. administration of MPTP (1 mg/nostril) or vehicle (saline). The animals were submitted to a battery of behavioral paradigms after MPTP administration that included olfactory discrimination (3rd day), social recognition (5th day), splash test (11th day) and open field (20th day) tasks. On the 21th day after MPTP injection, animals were euthanized and brain structures were collected for Western blotting assays. In order to assess the antioxidant status in the striatum, we evaluated the activity of antioxidant enzymes: CAT, GPx and GR through spectrophotometric methods. Tissues were collected 6 h after MPTP administration. The administration of MPTP induced impairments in olfactory discrimination ability and social recognition memory, anhedonic-like behavior and increased locomotor activity that was accompanied by a significant reduction of striatal tyrosine hydroxylase (TH) levels. Melatonin treatment protected selectively the olfactory deficits without changing the TH levels. Pre-treatment with vehicle plus MPTP and pre-treatment with melatonin plus vehicle induced an increase in CAT and GR activities in striatum. However, pre-treatment with melatonin prevented the MPTP-induced alterations in CAT and GR activities. These results reinforce and extend the potential of melatonin on treatment of PD and these findings can be explained, at least in part, by the known properties of melatonin to increase the cellular antioxidant defenses and act directly as free radical scavenger.

Disclosures: **J.M. Mack:** None. **T.M. Moura:** None. **C.L. Gonçalves:** None. **A.L. Dafre:** None. **M. Farina:** None. **R.D.S. Prediger:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.05/D12

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation for Parkinson's Disease

Title: Comparison of striatum and substantia nigra site of CDNF administration in rat 6-OHDA model of Parkinson's disease

Authors: *K. ALBERT, M. H. VOUTILAINEN, A. PANHELAINEN, R. K. TUOMINEN, M. AIRAVAARA, M. SAARMA;
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Abstract: Neurotrophic factors (NTF) regulate development of neurons, maintain adult neurons and have been shown to protect and repair damaged neurons in animals. Previous work has been done with NTFs and the neurodegenerative disorder Parkinson's disease (PD) in order to produce neurorestorative therapies, as none exist currently. Recently discovered cerebral dopamine neurotrophic factor (CDNF) has shown therapeutic potential in the rodent 6-OHDA and MPTP models of PD, showing it is a potent NTF to protect dopamine (DA) neurons and restore their function. Experiments were performed to test whether CDNF would have a neurorestorative effect on a 6-OHDA model of PD when given at different doses and injected to different locations in the rat brain. Two weeks after unilateral 6-OHDA lesion (2x10μg) to the striatum, recombinant human CDNF protein was injected into striatum, or substantia nigra, or both areas at varying doses. Amphetamine-induced rotations and cylinder test were used for motor asymmetry evaluation. Optical density of tyrosine hydroxylase fibres in the striatum and substantia nigra were measured to evaluate the severity of the lesion. Behavioural results and immunohistochemistry data indicated that the CDNF dose to the striatum (10μg) or substantia nigra (3μg) on their own were effective in restoring DA neurons. However, the combination of delivery into striatum and substantia nigra had an equally robust neurorestorative effect compared to the single injection into striatum or substantia nigra. Therefore, these injection paradigms work equally efficiently to restore DA neurons and bring about functional recovery.

Disclosures: **K. Albert:** None. **M.H. Voutilainen:** None. **A. Panhelainen:** None. **R.K. Tuominen:** None. **M. Airavaara:** None. **M. Saarma:** None.

Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson's Disease

Support: NIH-5P20GM103653-02

Title: Synergistic damage of commercially available environmental toxins in Parkinson's disease models

Authors: *E. M. JANEZIC, J. E. CAVINESS, S. G. KANDA, Y.-H. KIM;
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Abstract: Parkinson's Disease (PD) is the most common motor neurodegenerative disease. It is an age-related disease that affects 1-2% of the population over 60 years of age. The hallmark of PD pathology is the protein aggregation, known as Lewy Bodies, and loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc). Although several genetic mutations have been identified to cause PD, they only account for 5-10% of cases. The vast majority of cases are considered to be sporadic, or due to multifactorial reasons. Interestingly, there have been epidemiological studies that have shown that PD is more prevalent amongst farmers and rural populations. It has been suggested that exposure to pesticides and other environmental toxins may increase the risk of PD. In support of this notion, the pesticide rotenone is known to cause PD symptoms in flies and mice. Furthermore, it has been shown that the herbicide, paraquat, and the fungicide, maneb, can cause motor deficits individually, but cause synergistic damage when used together. Since this discovery, these pesticides have been banned in the US and EU. Here we propose that commercially available pesticides, when used together, can cause additive or synergistic damage in *in vitro* and mouse models of PD. We exposed rat dopaminergic N27 cells to commercially used pesticides such as acephate, alachlor, atrazine, chlorothalonil, diuron, glyphosate, imazethapyr, MCPA, and mecoprop at varying concentrations and measured cell viability using the MTT assay. Following single pesticide treatments, we measured cell viability when exposed to different combinations of these pesticides. Our results showed that only high concentrations of single pesticide treatments (e.g. $\geq$ 62.5 μM of chlorothalonil) caused a significant decrease in cell viability. However, when we examined the effect of the combined pesticides (starting with 14 combinations) at concentrations that did not show damage

individually, we identified eight combinations that caused additive damage and three that displayed synergistic damage. Our results suggest that exposure to multiple combinations of pesticides may cause dopaminergic toxicity and further lead to the PD pathology. Currently we are assessing the mechanism of action of toxicity *in vitro*, and analyzing the mouse brain, especially the striatum and the midbrain, after IP injections of a combination of pesticides for two weeks. The objectives of this study are to warn the public of the potential danger of using numerous combinations of pesticides and to impact on changes in policy for approval of pesticides. The results from this study may reveal potential causes for PD and reduce the prevalence of PD.

Disclosures: **E.M. Janezic:** None. **J.E. Caviness:** None. **S.G. Kanda:** None. **Y. Kim:** None.

Poster

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Topic: C.03. Parkinson's Disease

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Title: Pramipexole combined with non-viral transfection of BDNF recovers motor behavior in unilateral 6-OHDA-lesioned rats

Authors: L. R. QUINTERO[1], L. F. RAZGADO[1], A. J. ESPADAS[1], P. E. REYNA[1], A. SIERRA[1], V. ANAYA[3], I. JIMENEZ[1], D. MARTINEZ-FONG[1], *J. ACEVES[2]; [1]Physiol., [2]Ctr. Investigacion Del IPN, Mexico DF 07000, Mexico; [3]Morphology, Estudios Superiores de Iztacala UNAM, Mexico DF, Mexico

Abstract: The chronic (4 ½ months) and continuous (osmotic pump infusion) activation of dopamine D3 receptors combined with the selective and non-viral transfection of the BDNF gene to the remaining dopamine neurons restored nigrostriatal innervation and motor behavior in rats with 6-OHDA-induced degeneration of the nigral neurons (Razgado-Hernandez et al., 2015). Here we explored whether the chronic activation of D3R with Pramipexole (D3R-agonist widely used in clinics) orally administered combined with the BDNF transfection restores motor behavior in this model of PD. We used rats with unilateral lesions of the DA innervation induced

by intrastriatal 6-OHDA injected in three sites (7 µg/site). We evaluated gait (angle of the ankle), motor coordination (beam test), posture (rotarod), akinesia (cylinder test) and muscle rigidity (EMG). Pramipexole was given twice (1 mg/Kg) for 5 months and BDNF was transfected into the DA neurons using the NT-Poliplex (Martinez-Fong et al., 2012), a method that exclusively transfect DA neurons. The combined treatment restored gait, motor coordination and posture and eliminated akinesia and muscle rigidity. The recovery of motor behavior persisted even three months after the end of the treatment, suggesting a trophic effect. The recovery was associated with a partial (about 40 %) recovery of the striatal DA innervation (judged by densitometry) and a partial (about 30%) recovery of the number of DA neurons of the pars compacta. The recovery was also associated with a high (about 80%) expression of the BDNF gene (or protein) and with the expression of BrdU (suggesting neurogenesis) in the TH(+) neurons of the pars compacta. Neither Pramipexole nor BDNF alone recovered motor behavior. Thus, the BDNF transfection to dopamine neurons associated with oral Pramipexole offers a promising strategy for the treatment of Parkinson´s disease.

Disclosures: **L.R. Quintero:** None. **L.F. Razgado:** None. **A.J. Espadas:** None. **P.E. Reyna:** None. **A. Sierra:** None. **V. Anaya:** None. **I. Jimenez:** None. **D. Martinez-Fong:** None. **J. Aceves:** None.

Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson’s Disease

Support: The State of Indiana

Title: Body and facial pain in an animal model of Parkinson’s diseases and its possible mechanism and treatment

Authors: *A. TRUONG[1], G. G. ACOSTA[2], R. SHI[2];
[1]BME, [2]Purdue Univ., West Lafayette, IN

Abstract: Parkinson’s disease (PD) is a neurodegenerative movement disorder in which dopaminergic neurons are progressively lost from the brain. The classic symptoms of PD consist of tremor, rigidity, bradykinesia, and postural instability. However, there are also non-motor symptoms of PD that may be even more troublesome than the motor abnormalities. One of the important and under-appreciated complaints among these non-motor symptoms is pain, which is

estimated to be present in more than half of PD patients in various forms and locations. Using a bilateral 6-OHDA/DSP-4 lesion model of PD in rats, we show that parkinsonian rats had increased mechanical sensitivity in the von Frey test of allodynia in hind paw compared to sham-lesioned rats, indicating an increased mechanical pain response in the body extremities in this animal PD model. We also performed the periorbital tactile sensitivity test in these rats to determine the extent of headache and facial pain. Lesioned rats exhibited reduced periorbital pain thresholds compared to sham-lesioned rats indicating increased periorbital sensitivity in parkinsonian rats. While the mechanisms of such neuropathic pain in PD have not been investigated in detail, we have discovered that acrolein, a highly reactive unsaturated aldehyde that has previously been shown to be involved in establishing and maintaining neuropathic pain in spinal cord injury (SCI), was elevated in the lesioned rats. Furthermore, acrolein injection could produce motor behavioral deficits that resemble those associated with the 6-OHDA rats, indicating a probable pathological role of acrolein in this animal model. Interestingly, administration of the acrolein scavenger hydralazine after manifestation of both body and facial pain can reduce acrolein levels and more importantly, mitigate pain sensitivity of parkinsonian rats in both types of pain, in addition to alleviating motor deficits. Acrolein injection to the brain has also produced noticeable neuroinflammation, a process well-known to contribute to neuropathic pain, further supporting a possible role of acrolein in PD pain. Taken together, we have established a model of quantifying neuropathic pain in both body extremities and in facial area associated with a well-established 6-OHDA animal PD model. Further, we have gathered initial evidence that a known pain inducer, acrolein, likely plays an algesic role in pain in a 6-OHDA rat model of PD. Furthermore, anti-acrolein treatment appears to be an effective strategy for pain mitigation in this model.

Disclosures: **A. Truong:** None. **G.G. Acosta:** None. **R. Shi:** None.

Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson’s Disease

Support: Tenovus Scotland

Title: Symptomatology in Parkinson’s disease: a translational behavioural study in two different 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse models

Authors: *M. SANTORO[1], V. MELIS[2], P.-H. MOREAU[2], J. V. FORRESTER[3], G. RIEDEL[3], P. TEISMANN[3];
[1]Col. of Life Sci. and Med., [2]Sch. of Med. and Dent., [3]Sch. of Med. Sci., Univ. of Aberdeen, Aberdeen, United Kingdom

Abstract: Background - Nigrostriatal dopaminergic neuronal loss and presence of Lewy bodies are two pathological hallmarks of Parkinson's disease (PD). Intoxication with MPTP is the only example of toxin induced neurodegeneration recapitulating different hallmarks of the disease in human, primates and rodents. PD symptomatology presents with motor and non-motor manifestations. Diagnosis of the disease is based on the onset of motor symptoms, whilst non-motor manifestations are often prodromal to the diagnosis of the disease and observed in 60 % of PD patients. **Objective -** Complete behavioural characterization of two MPTP mouse models of PD has been performed to identify behavioural endpoints valid as translational biomarkers that can discriminate at a sufficiently robust level between saline controls and MPTP lesioned mice. **Methods -** Male C57BL/6J mice ten weeks old were injected i.p. with a sub-acute (30 mg/kg once a day for 5 consecutive days) and acute (4 injections of 20 mg/kg each 2 hours) regimen of the neurotoxin MPTP, control mice received an equivalent volume of saline. Eight behavioural tests were performed chronologically: light-dark box (anxiety), Rotarod, balance beam and CatWalk (gait analysis), PhenoTyper (homecage activity, circadian rhythm), olfactory discrimination and sucrose preference (depression), and Barnes maze (cognition). Behavioural readouts are correlated with dopaminergic neurons in the substantia nigra (stereological cell counting) and, dopaminergic fibres in the striatum (optical density quantification). **Results -** Differences in motor-coordination were detected with balance beam test (latency to traverse) in the sub-acute model, but no fatigue-induced phenotype was revealed in the Rotarod. In the sub-acute MPTP group, marked hyperactivity was observed in olfactory discrimination test (path length), which also affected the habituation to a novel environment (path length per time bin, PhenoTyper). By contrast, acute MPTP administration severely disrupted the animal's gait quantified with CatWalk (stance, stride length, and swing speed). Again, the sub-acute but not the acute MPTP injection regimen yielded a mild cognitive deficit (Barnes maze). **Conclusions -** We have identified behavioural translational biomarkers for PD symptomatology in two MPTP mouse models; greater impairment was achieved using the sub-acute dosing regimen, which affected all disease-relevant symptom domains. This raises two issues: i) Does sub-acute MPTP treatment also conform with the molecular anomalies underlying PD? ii) How does sub-acute MPTP compare to a chronic administration protocol in terms of symptoms?

Disclosures: M. Santoro: None. **V. Melis:** None. **P. Moreau:** None. **J.V. Forrester:** None. **G. Riedel:** None. **P. Teismann:** None.

Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson's Disease

Support: Molecular Medicine Ireland Clinical and Translational Research Scholarship

Title: Characterisation of cognitive dysfunction in an alpha-synuclein rodent model of Parkinson's disease

Authors: *E. K. DOLAN, Y. M. NOLAN, A. M. SULLIVAN;
Anat. and Neurosci., Univ. Col. Cork, Cork, Ireland

Abstract: Background: Viral vector-mediated overexpression of α-synuclein in rodent brains *in vivo* is a newly-developed animal model of Parkinson's disease (PD). It has been shown to reproduce many of the clinical features of PD, including nigral dopaminergic neuron degeneration, decreased striatal dopamine levels and significant motor impairment. In addition to the characteristic motor symptoms seen in PD, cognitive dysfunction such as depression, dementia and dysexecutive syndrome can manifest as the disease progresses. Our study aims to investigate and characterise late-stage cognitive and motor dysfunction using the α-synuclein rat model of PD. **Methods:** Adult male Sprague Dawley rats received into the substantia nigra either unilateral ($3.1x10^8$gc/3µl) or bilateral (two injections of $1.6x10^8$gc/3µl) injection of an adeno-associated viral vector serotype 2/6 overexpressing either human wildtype α-synuclein (n=10 per group) or GFP (n=8 per group). An additional cohort of control animals did not undergo surgery (n=8). Animals underwent a series of motor and cognitive tests at various time points throughout the experiment. **Results:** α-synuclein-lesioned animals exhibited significant motor dysfunction in the stepping test (p=0.001) 20 weeks post-surgery. Early results indicate the onset of cognitive deficits in bilaterally-injected α-synuclein animals at 30 weeks post-surgery, where they showed significant impairment in performance in an olfactory discrimination task and a conditioned taste aversion protocol. Interestingly, there was no difference across the groups after measuring spontaneous alternations in a Y maze. Further immunohistochemical and HPLC analysis is being carried out to examine the progression of the neurodegeneration and α-synuclein pathology in various brain regions. **Future work:** An increasing amount of evidence points to beneficial effects of exercise on both motor and cognitive symptoms in PD, and we are currently running further studies to examine the protective effects of exercise on cognitive dysfunction seen in the α-synuclein model.

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Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson's Disease

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The Graduate Center, CUNY

Title: *In vivo* μPET imaging of neuroinflammation in a rat model exhibiting Parkinsonian-like pathology

Authors: *C. CORWIN[1,3], M. NUNEZ-SANTOS[1], A. NIKOLOPOULOU[4], Y. KANG[4], S. VALLABHAJOSULA[4], P. SERRANO[2], J. BABICH[4], M. FIGUEIREDO-PEREIRA[1]; [1]Biol., [2]Psychology, Hunter College, CUNY, New York, NY; [3]The Grad. Center, CUNY, New York, NY; [4]Radiology, Weill Cornell Med. Col., New York, NY

Abstract: Chronic neuroinflammation is a critical factor in the pathogenesis of Parkinson's disease (PD). *In vivo* detection of the early stages of brain inflammation could provide an optimal strategy to establish the most effective time for therapeutic intervention prior to a point of no return in PD. To test *in vivo* brain imaging of neuroinflammation in PD, we established a mouse model of inflammation with subchronic microinfusion of prostaglandin J2 (PGJ2) into the nigral/striatal area of adult FVB male mice. PGJ2 is a highly toxic endogenous product of inflammation that impairs the ubiquitin/proteasome pathway and triggers the accumulation/aggregation of ubiquitinated proteins and caspase activation. Thus, PGJ2 induces many of the pathological processes involved in PD. The PGJ2-treated mice exhibited a dose-dependent (a) reduction of dopaminergic neurons in the substantia nigra pars compacta (SNpc) with little damage to local GABAergic interneurons, (b) activation of microglia and astrocytes, (c) neuronal Lewy-like bodies and (d) impairment of gait and balance mimicking PD behavior. We also evaluated microglia activation *in vivo* by performing μPET imaging with [11C](R)PK11195. The latter binds to the mitochondrial translocator protein (TSPO) that is overexpressed upon microglia activation. We observed a qualitative increase in [11C](R)PK11195 signal in the SNpc of PGJ2-treated compared to DMSO-treated mice, confirming that PGJ2 induces microglia activation. To improve resolution and image quality of

μPET imaging, we are establishing a PGJ2-induced rat model of inflammation relevant to PD. Adult Sprague Dawley male rats received four unilateral injections of PGJ2 or DMSO into the right SN. Behavioral assays measuring asymmetry and hypokinetic symptoms and μPET imaging were performed over time. The intact contralateral side served as the basal level for each rat. To provide a correlation between microglia and astrocyte activation in brain inflammation, we used the microglia marker [11C](R)PK11195 and the aromatase inhibitor [11C]vorozole in μPET imaging. Vorozole was used as a marker for astrocyte activation, as it binds to aromatase that is rapidly up-regulated in astrocytes upon brain injury. Aromatase induces estrogen synthesis in the brain. All μPET imaging was compared to immunohistochemistry analyses including aromatase expression. The use of both TSPO and aromatase tracers to image neuroinflammation in the same biological model is novel. Moreover, *in vivo* imaging of both astrocyte and microglial activation is critical to understanding the role of inflammation and its detection in neurodegenerative diseases such as PD.

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Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson’s Disease

Title: Decoupled involvement of the dorsolateral striatum and of the pre-frontal cortex in depressive symptoms in 6-hydroxydopamine rat model of Parkinson’s disease

Authors: *F. C. MATHEUS[1], D. RIAL[1], J. I. REAL[2], C. LEMOS[2], R. N. TAKAHASHI[1], L. J. BERTOGLIO[1], R. A. CUNHA[2], R. D. PREDIGER[1];
[1]Pharmacol., Federal Univ. of Santa Catarina, Florianópolis, Brazil; [2]Univ. of Coimbra, Coimbra, Portugal

Abstract: The dorsolateral striatum (DLS) is involved with motor and non-motor behavioral functions and undergoes extensive dopaminergic degeneration in Parkinson`s disease (PD). This dopaminergic neurodegeneration also disrupts other brain areas as the prefrontal cortex (PFC), which has been associated with the appearance of non-motor symptoms of PD. Using behavioral, neurochemical and electrophysiological approaches, we evaluated the structural and temporal dissociation between the role of the DLS and of the PFC in the appearance of depressive-like

behaviors in rats submitted to bilateral DLS lesions with the neurotoxin 6-hydroxydopamine (6-OHDA). The 6-OHDA generated a partial dopaminergic nigrostriatal lesion with motor impairments being observed only at the highest tested dose (20 μg/site). However, the lower 6-OHDA doses (5 and 10 μg/site) did not induce such impairments. Anhedonic-like behaviors were observed in the splash and sucrose consumption tests at 7 days after 6-OHDA lesion. In contrast, helplessness behaviors, as evaluated in the forced swimming and social interaction tests only emerged 21 days after 6-OHDA lesion when anhedonia was no longer present. These temporally dissociated behavioral alterations were coupled to temporal- and structure-dependent alterations in dopaminergic markers such as dopamine D1 and D2 receptors and dopamine transporter (DAT), leading to altered dopamine sensitivity in DLS and PFC circuits, evaluated electrophysiologically. Pharmacological treatments during 7 or 21 days with fluoxetine (10 mg/kg), bupropion (10 mg/kg) or quinpirole (0.1 mg/kg) prevented the onset of anhedonic-like and helplessness-like behaviors. These results provide the first demonstration of a dissociated involvement of the DLS and PFC in anhedonic- and helplessness behaviors in an animal model of PD, which was related with temporal fluctuations in density and functionality of dopaminergic receptors. Leading to altered dopaminergic system sensitivity in these two brain structures. This study sheds new light to the duality between different types of depressive symptoms in PD.

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Poster

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Topic: C.03. Parkinson’s Disease

Title: Role of bdnf/trkb pathway in l-dopa-induced dyskinesia in mice

Authors: *A. PELOSI[1,2,3], J.-C. CORVOL[3,4,5,6], B. XU[7], J.-A. GIRAULT[1,2,3], D. HERVÉ[1,2,3]; [1]Inst. du Fer a Moulin, Paris, France; [2]Inserm UMR-S 839, Paris, France; [3]Univ. Pierre et Marie Curie-Paris 6, Paris, France; [4]Inserm, UMR-S 1027, ICM, Pitié-Salpêtrière Hosp., Paris, France; [5]CNRS, UMR 7225, Paris, France; [6]Assistance Publique Hôpitaux de Paris, Inserm, Clin. Investigation Ctr. (CIC-1422), Pitié-Salpêtrière Hosp., Paris, France; [7]Dept. of Neurosci., The Scripps Res. Inst., Jupiter, FL

Abstract: L-DOPA-induced dyskinesia (LID) is a frequent adverse side effect of the treatment for Parkinson’s disease (PD). Understanding the mechanisms underlying the development of

these motor disorders is needed to reduce or prevent them. We investigated the role of TrkB receptor in L-DOPA-induced dyskinesia in hemi-parkinsonian mice rendered dyskinetic by chronic L-DOPA administration. L-DOPA treatment specifically increased TrkB receptor mRNA and protein levels in the dopamine-depleted dorsal striatum as compared to the contralateral unlesioned striatum or to the striatum of sham-operated animals. The dopamine depletion alone or acute L-DOPA treatment did not significantly increase TrkB protein levels. In addition to increasing TrkB protein levels, chronic L-DOPA treatment activated TrkB receptor leading to increased tyrosine phosphorylation of TrkB and PLCγ. These results indicate that BDNF/TrkB-dependent pathway is highly up-regulated by chronic L-DOPA treatment in dopamine-depleted striatum and could be involved in the development of abnormal movements, typical of dyskinesia. In support of this hypothesis, we found that targeted invalidation of TrkB gene in the striatum reduced the development of L-DOPA-induced dyskinesia. Our study suggests that interfering with BDNF/TrkB signalling could be a valuable approach to reduce the side effects of L-DOPA treatment in Parkinsonian patients.

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Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson’s Disease

Title: Development of an early stage model of Parkinson's disease

Authors: ***K. FARMER**[1], C. RUDYK[1], T. FORTIN[1], C. A. SMITH[1], N. PROWSE[1], J. C. SMITH[2], S. P. HAYLEY[1];
[1]Neurosci., [2]Chem., Carleton Univ., Ottawa, ON, Canada

Abstract: Parkinson’s disease (PD) is a devastating age-related neurodegenerative disease that affects primarily the dopamine (DA) neurons of the nigrostriatal pathway and results in debilitating motor, and sometimes, emotional and cognitive deficits. Current treatments only manage symptom severity, and there exists no treatment that is able to reverse or even appreciably slow down the neurodegenerative course of the disease. With this in mind, there is an urgent need to promote neuroregenerative or recovery processes. Additionally, it is our belief that therapeutic intervention must occur in the early stages of the disease. As such, the following studies demonstrate how administration of low-dose 6-hydroxydopamine (6-OHDA) can be used

to model the early stages of PD. Furthermore, we found that the neurotrophic cytokines erythropoietin (EPO) and granulocyte macrophage-colony stimulating factor (GM-CSF) may have important therapeutic potential for PD. From our series of experiments, we found that low doses of 6-OHDA administered into the anterior dorsal striatum were able to induce a mild partial lesion that progressed over 30 days with no significant loss of DA cells in the substantia nigra (SNc). Additionally, 21 days after neurotoxin administration there was a decrease in structural phosphatidylcholine lipids and a large increase in inflammatory and immune system related lysophosphatidylcholines. We also observed motor dysfunction that progressed over time in a dose-dependent manner that was related to overall striatal DA loss. Finally, we have shown that treatment with the trophic cytokines EPO or GM-CSF induced striatal re-innervation when administered long after the neurotoxic insult. Taken together, low doses of 6-OHDA were able to induce some of the hallmark behavioural and pathophysiological markers of early stage PD. Additionally, treatment with GM-CSF and EPO may provide novel therapeutic agents aimed at fostering neural recovery.

Disclosures: **K. Farmer:** None. **C. Rudyk:** None. **T. Fortin:** None. **C.A. Smith:** None. **N. Prowse:** None. **J.C. Smith:** None. **S.P. Hayley:** None.

Poster

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Support: Jerry T. and Glenda G. Jackson Fellowship in Parkinson’s Research to the University of Arizona

Title: Long-term effect of sub-anesthetic ketamine infusion in reducing L-DOPA-induced dyskinesias

Authors: **M. J. BARTLETT**[1], L. M. LEPOIDEVIN[1], R. M. JOSEPH[1], K. L. PARENT[2], N. D. LAUDE[2], L. B. LAZARUS[2], M. L. HEIEN[2], M. ESTEVEZ[3], S. J. SHERMAN[1], *T. FALK[1]; [1]Dept. Of Neurol., Univ. of Arizona Col. of Med., Tucson, AZ; [2]Chem. & Biochem., Univ. of Arizona, Tucson, AZ; [3]Neurometrica, LLC, Eugene, OR

Abstract: Low-dose sub-anesthetic ketamine infusion treatment has led to a long-term reduction of treatment-resistant depression and posttraumatic stress disorder (PTSD) symptom severity, as well as reduction of chronic pain states, including migraine headaches. Ketamine also is known

to change oscillatory electric brain activity. Repurposing drugs that have already been proven safe in humans has the potential to offer new therapies at a fraction of the time required to develop new drug treatments. One commonality between migraine headaches, depression, PTSD, Parkinson's disease (PD) and L-DOPA-induced dyskinesia (LID) is hypersynchrony of electric activity in the brain, including the basal ganglia. An effective treatment of LID to extend the useful lifetime of L-DOPA treatment is a critical unmet need in PD therapy. Therefore, we investigated the use of low-dose ketamine in the treatment of PD and LID. We show a long-term therapeutic effect of low-dose ketamine infusion (0.15 - 0.3 mg/kg/hr for 72 hrs) from five PD patient case studies identified by a retrospective chart review (reduced dyskinesia, improved on time, and reduced depression). Additionally, in the standard preclinical rodent model of LID (using 7 mg/kg L-DOPA to prime unilaterally 6-hydroxydopamine-lesioned rats), ketamine (5 - 20 mg/kg) led to long-term dose-dependent reduction of abnormal involuntary movements (AIMs), only when sub-anesthetic low-dose ketamine was given for ten hrs (n=5) and not with a single acute (n=10) low-dose ketamine injection (one way ANOVAs vs baselines, followed by Tukey post hoc tests). To mimic the patient infusion for the 10 hr paradigm ketamine was injected 5 x i.p. two hrs apart, 7 mg/kg L-DOPA was co-injected at the 5th injection, and then the AIMs scores were evaluated. For the ketamine 'infusion' doses the anti-dyskinetic effects lasted days to weeks past the 'infusion' day. A significant anti-dyskinetic effect of the 20 mg/kg ketamine 'infusion' paradigm remained apparent 55 days later, and an additional 10 mg/kg infusion did not show sensitization. To gather pharmacokinetic data tail vain blood was collected at time points during and up to 10 days post a 15 mg/kg 'infusion' protocol, and levels of ketamine and the major metabolite norketamine are currently analyzed (n=10). This novel use of low-dose sub-anesthetic ketamine infusion could lead to fast clinical translation, and since depression and comorbid pain states are critical problems for many Parkinson's disease patients could open up the road to a new dual therapy for patients with L-DOPA-induced dyskinesias. Support: Jerry T. and Glenda G. Jackson Fellowship in Parkinson's Research to the University of Arizona.

Disclosures: **M.J. Bartlett:** None. **L.M. LePoidevin:** None. **R.M. Joseph:** None. **K.L. Parent:** None. **N.D. Laude:** None. **L.B. Lazarus:** None. **M.L. Heien:** None. **M. Estevez:** None. **S.J. Sherman:** None. **T. Falk:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.16/D23

Topic: C.03. Parkinson's Disease

Support: Swedish Research Council

Title: An age- and time-course study of the responses to nigrostriatal lesions and dopaminergic treatments in mice

Authors: *F. BEZ[1], V. FRANCARDO[2], M. A. CENCI[2];
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Abstract: Mice with 6-OHDA lesions are widely used as a model to study L-DOPA-induced dyskinesia (LID), a major therapy complication in Parkinson´s disease. These studies generally utilize young adult mice with relatively acute 6-OHDA lesions. However, studies of LID in genetic mouse models of PD utilize older animals where a parkinsonian-like phenotype has become manifest (Brehm et al. Mol Neurobiol 2014). Ageing affects several pathways of neuroplasticity in the brain, and may therefore impact on an animal´s response to chronic dopaminergic treatments. We have therefore undertaken to compare behavioral and histopathological phenotypes at short- or long-term intervals after 6-OHDA lesions in mice. At 2 months of age, mice sustained unilateral intrastriatal injections of 6-OHDA (2 µl x 2, cf. Francardo et al. Neurobiol Dis 2011). Baseline motor deficits (cylinder test and overall activity) and responses to L-DOPA and apomorphine were evaluated at 3 weeks and 21 months after the 6-OHDA lesion. A video-based methodology was used for simultaneous quantification of abnormal involuntary movements (AIMs) and motor stereotypies, as well as other behavioral items (e.g. horizontal and vertical activity, rearings and rotations). Striatal monoaminergic innervation densities were studied immunohistochemically. A three-weeks treatment with ascending doses of L-DOPA (12-48 mg/kg/day) was used to induce AIMs. This treatment induced severe axial, limb and orolingual AIMs when given 3-5 weeks after the 6-OHDA lesion. In contrast, the same treatment did not induce any dyskinetic behavior when given 21 months post-lesion. However, these old mice exhibited stereotypic behaviors in response to apomorphine (0.25-3.0 mg/kg). Significant differences between the two ages/post-lesion intervals were detected also on measures of rotational behavior, vertical activity, and stereotypies. The immunohistochemical analysis revealed prominent sprouting of both dopaminergic and serotonergic fibers in the lateral striatum ipsilateral to the lesion at 21 months. This is the first study to examine the profiles of behavioral deficits and responses to dopaminergic treatments in 6-OHDA-lesioned mice that reached an advanced age. We report dramatic differences in the response to L-DOPA between young and old mice, possibly related to the occurrence of a pronounced compensatory axon sprouting in the striatum at long post-lesion intervals. These results are part of a larger ongoing study, and we are now studying the effects of 6-OHDA lesions in already old mice.

Disclosures: F. Bez: None. **V. Francardo:** None. **M.A. Cenci:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.17/D24

Topic: C.03. Parkinson's Disease

Support: JSPS (Japan) to KH, YK, and JLZ

Title: Cineradiographic analysis of respiratory movements in a murine model mimicking different stages of Parkinson's disease

Authors: *P. SALES DE CAMPOS[1], L. R. S. M. KAWAMURA[1], K. HASEGAWA[2], Y. KUMEI[3], J. L. ZEREDO[1,3];
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder that leads to degeneration of dopaminergic neurons of the substantia nigra. Respiratory disorders occur in most patients with PD, causing a decrease in compliance of the rib cage by increased stiffness, reduced air volume and flow rates of inspired air, fatigue and incoordination of diaphragmatic and accessory muscles. Furthermore, the mechanical restriction of diaphragmatic mobility promotes an imbalance in the ventilation/perfusion ratio, causing hypoventilation in pulmonary ventilation-dependent areas. It has been proposed that such changes are secondary to changes in posture and osteoarticular degeneration, leading to an alteration in the spinal axis that in turn could affect breathing mechanics. Nevertheless, clinical studies have failed to show a clear relationship between respiratory symptoms and the stage of the disease. In this study, we aimed of testing changes the in respiratory funcion in relation to the degree of nigrostrial degeneration. We employed a murine models of mild and severe hemi-Parkinson's Disease. C57BL/6J mice were used. Under surgical anesthesia, PD mice received an injection of different doses of 6-OHDA solution to the right nigro-striatal pathway though a stereotaxically driven microsyringe. These two groups were compared to control mice, which received an injection of saline under the same conditions. Two weeks after surgery, all mice had their respiratory movements recorded by video x-rays without any restraint. Parameters of respiratory function (diaphragm displacement, costophrenic angle, distance between costophrenic angles, and respiratory rate) showed a different patterns of alterations between mild and severe PD. These results suggest a different nature of respiratory alterations in early vs. late stage PD.

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Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson's Disease

Support: IAPP Grant: FP7-PEOPLE-2013-IAPP GA N 612275

Parkinson's UK Innovation Grant: K-1408

Title: The neuroprotective effect of GDNF Family Ligand Mimetics in unilateral 6-hydroxydopamine-model of Parkinson´s disease in rats

Authors: *J.-M. RENKO[1], Y. SIDOROVA[1], M. VOUTILAINEN[1], J. SAKKI[1], M. KARELSON[2], M. SAARMA[1], R. K. TUOMINEN[1];
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Abstract: Background Neurotrophic factors (NTFs) are secreted proteins supporting the survival and regeneration of neurons. Therefore, NTFs might be beneficial for the treatment of neurodegenerative disorders such as Parkinson´s disease (PD). Glial cell line-derived neurotrophic factor (GDNF) has shown neuroprotective and neurorestorative effects on dopaminergic neurons in animal models of PD. As GDNF is a protein, there are several problems to be solved before it can be used clinically e.g. i) it is expensive to produce in quantities sufficient for clinical use, ii) the effects and adverse effect profile of GDNF have been contradictory in clinical studies and iii) systemic delivery of the protein is problematic. GeneCode Ltd. has developed small molecular weight compounds, which activate GDNF Family Ligand (GFL) receptors (GFRα-RET) and downstream signaling pathways similar to GFL proteins both *in vitro* and *in vivo*. The aim of this study was to test the neuroprotective effect of one GFL mimetic (GFLM) *in vivo* in the 6-hydroxydopamine (6-OHDA) model of PD. **Methods** In the first stereotaxic operation 6-OHDA (16 µg/4 µl) was injected into the left *striatum* of male Wistar rats (n=6-13/treatment group). One hour later osmotic mini-pumps were implanted s.c. and cannulas were placed into the left *striatum*. GFLM (1 µg/24h, 3 µg/24h or 6 µg/24h), GDNF (3 µg/24h) or vehicle (propylene glycol or phosphate buffered saline) was infused for 7 days at the rate of 0.5 µl/h. The mini-pumps were removed in the second stereotaxic operation. Amphetamine-induced (2.5 mg/kg, i.p.) rotational behavior was tested 2, 4 and 6 weeks after the

6-OHDA lesion. Forelimb asymmetry test was performed at 4 weeks time point. After the last rotational behavior test rats were perfused and coronal brain sections were made. Tyrosine hydroxylase (TH) immunostaining will be analyzed from *substantia nigra* and *striatum.* **Results** The GFLM (1, 3 and 6 µg/24h) dose-dependently reduced amphetamine-induced rotations four and six weeks after the 6-OHDA lesion. The effect was comparable to what was observed in animals treated with GDNF protein (3 µg/24h). The analyses of the number of TH-positive cells in *substantia nigra pars compacta* and the optical density of TH-positive fibers in *striatum* are still ongoing. **Conclusions** The results suggest that the GFLM tested here has similar activity as GDNF protein in unilateral 6-OHDA-model of PD in rats. Thus, small molecules that activate GFL receptors and their signaling pathways may have potential as a new disease modifying therapy of PD. Because of small molecular weight, GFLMs could be more suitable for pharmaceutical industry and for clinical use than NTF proteins.

Disclosures: J. Renko: None. **Y. Sidorova:** None. **M. Voutilainen:** None. **J. Sakki:** None. **M. Karelson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of patent which is owned by GeneCode Ltd.. Other; GeneCode Ltd. and University of Helsinki have a research agreement. **M. Saarma:** 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.; Project funding: Industry-Academia Partnerships and Pathways (IAPP)- Marie Curie Actions, Project funding: Parkinson´s UK. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of the patent which is owned by GeneCode Ltd.. Other; GeneCode Ltd. and University of Helsinki have a research agreement. **R.K. Tuominen:** None.

Poster

676. Parkinson's Disease: Rodent Models II

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Program#/Poster#: 676.19/D26

Topic: C.03. Parkinson’s Disease

Support: NS059921

Title: Zolpidem ameliorates motor impairments in unilateral 6-OHDA rodent model of Parkinson's disease

Authors: *R. ASSINI, E. D. ABERCROMBIE;
Ctr. for Mol. & Behavioral Neurosci., Rutgers Univ. - Newark, Newark, NJ

Abstract: Nodes within the basal ganglia, such as the globus pallidus external segment (GPe) and subthalamic nucleus (STN), have been shown to exhibit increased firing as well as synchronous bursting activity entrained to excessive cortical beta oscillations in unilateral 6-OHDA rodent models of Parkinson's disease (PD). Ionotropic GABA-A receptors are expressed ubiquitously throughout the basal ganglia, but exhibit disparate subunit expression in a nucleus-specific manner. The distribution of alpha subunit types between basal ganglia nuclei confers differential sensitivity to benzodiazepine-like compounds between each node of the circuit. Zolpidem is an imidazopyridine that acts as a positive allosteric modulator of GABA-A receptors, potentiating iPSCs with binding selectivity for receptors expressing the alpha-1 subunit. Coincidentally, the nuclei expressing the alpha-1 subunit within the basal ganglia are also those that have been shown to have increased bursting activity in a dopamine-depleted state. Using rotarod and cylinder test, we investigated whether differential expression of the alpha-1 subunit between basal ganglia nuclei indicates that zolpidem may be a potential non-dopaminergic therapeutic option in the treatment of motor symptoms of PD. Male Sprague-Dawley rats were tested for baseline/pre-lesion performance on the tasks, then lesioned using unilateral infusion of 6-OHDA into the right medial forebrain bundle (MFB). Animals with confirmed lesions were subsequently tested for post-lesion performance on the tasks, then tested for performance following acute intraperitoneal (IP) injection of zolpidem (0.1, 0.25, 0.5 mg/kg) or vehicle. Using the rotarod balance beam task, an improvement (~60%) in performance was found following acute IP administration of 0.1 mg/kg zolpidem compared to undrugged post-lesion performance as well as vehicle. Using the cylinder test, it was found that acute IP administration of 0.1 mg/kg zolpidem reduced unilateral forelimb use bias compared to post-lesion performance as well as vehicle. From this data, it can be concluded that zolpidem may be a potential therapeutic option in the treatment of motor symptoms of PD.

Disclosures: R. Assini: None. **E.D. Abercrombie:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

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Topic: C.03. Parkinson's Disease

Support: NSF CAREER-1351112

Utah Science, Technology, and Research Initiative

Title: Subthalamic deep brain stimulation reduces pathological information transmission to the thalamus in a rat model of Parkinsonism

Authors: *C. ANDERSON, A. DORVAL;
Univ. of Utah, Salt Lake City, UT

Abstract: The degeneration of dopaminergic neurons in the substantia nigra pars compacta leads to parkinsonian motor symptoms via changes in electrophysiological activity throughout the basal ganglia. High- frequency deep brain stimulation (DBS) partially treats these symptoms, but the mechanisms are unclear. We hypothesize that motor symptoms of Parkinson's disease are associated with increased information transmission between the substantia nigra reticulata (SNr) in the ventral basal ganglia and the ventral anterior thalamus (VA), and that therapeutic DBS treats these symptoms by reducing this extraneous information transmission. We tested these hypotheses in a unilateral, 6-hydroxydopamine- lesioned rodent model of hemiparkinsonism. In the hemiparkinsonian condition, information transfer between SNr and VA was significantly increased in both the orthodromic and antidromic directions, and these changes were reversed by behaviorally therapeutic DBS. Omnidirectional information increases in the parkinsonian state underscore the detrimental nature of that pathological information, and suggest a loss of information channel independence. Therapeutic DBS reduced that pathological information, suggesting an effective increase in the number of independent information channels. We interpret these data with a model in which pathological information and fewer information channels diminishes the scope of possible motor activities, driving parkinsonian symptoms. In this model, DBS restores information-channel independence by eliminating or masking the parkinsonism associated information, and thus enlarges the scope of possible motor activities, alleviating parkinsonian symptoms.

Disclosures: C. Anderson: None. **A. Dorval:** None.

Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson’s Disease

Support: MJFF rapid response initiative award

Title: Vagus nerve stimulation paired with motor training does not improve forelimb function or strength in 6-hydroxydopamine-lesioned rats

Authors: *A. NGUYEN[1], A. RUIZ[1], S. HAYS[2], M. KILGARD[2], R. RENNAKER[2];
[2]Brain and Behavioral Sciences, Neurosci., [1]Univ. of Texas At Dallas, Richardson, TX

Abstract: Vagus nerve stimulation (VNS) is a safe, tolerable treatment that has been shown to drive powerful, long-lasting plasticity especially when paired with somatosensory inputs. VNS is being investigated as an adjunct treatment to physical rehabilitation and has been shown to improve recovery rates in models of stroke, traumatic brain injury, and tinnitus. VNS paired with forelimb motor training has been shown to increase the forelimb representation area in primary motor cortex (M1). Increases in motor map size and reorganization of motor map topography after neurological injury correlate with functional recovery. Parkinson's Disease is a neurodegenerative disorder involving motor dysfunction and maladaptive plasticity in motor circuitry. To model parkinsonism in rats, researchers use a neurotoxin called 6-hydroxydopamine (6-OHDA) to cause selective depletion of catecholaminergic cells, particularly dopamine cells in the substantia nigra. Administration of 6-OHDA in rats causes motor dysfunction and has been shown to reduce the size of motor maps in M1. The aims of this study were to investigate whether VNS-paired with motor training can induce plasticity in M1 of 6-OHDA lesioned rats, whether VNS can improve motor performance on multiple assays of motor function, and whether changes in motor map size and topography correlate with functional recovery or force generation in these rats. To investigate this, rats were trained to proficiency on an automated force generation task, in which the goal for the rats was to pull a stationary handle with a predetermined amount of force to receive a food pellet reward. Rats were then administered 6-OHDA unilaterally into four sites within the striatum and implanted with a vagus nerve stimulation cuff on the left vagus nerve. After 1 week of post surgical recovery, rats performed the force generation task as physical rehabilitation over the course of 6 weeks with ("VNS Rehab" group) or without VNS ("Rehab Only" group). In the VNS Rehab group, VNS was delivered as brief pulses of electrical current during correct trials (pull attempts that exceeded the 120g force threshold required for a hit and subsequent delivery of a food pellet). Cylinder and forelimb placing tests were employed before and 1 week after the induction of the lesion, and at the end of therapy. At all timepoints during therapy, post lesion performance was significantly impaired when compared to pre-lesion levels, however there was no difference between the groups on either a measure of hit rate or force generation.

Disclosures: **A. Nguyen:** A. Employment/Salary (full or part-time):; Texas Biomedical Device Center. **A. Ruiz:** A. Employment/Salary (full or part-time):; Texas Biomedical Device Center. **S. Hays:** None. **M. Kilgard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Microtransponder. **R. Rennaker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vulintus, LLC.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.22/D29

Topic: C.03. Parkinson's Disease

Support: Selma Schottenstein Harris Lab for Research in Parkinson's

Gardner Family Center for Parkinson's Disease and Movement Disorders

Title: Non-motor behavioral alterations and cell degeneration in extranigral brain regions of the DJ-1 knockout rat

Authors: *T. L. KYSER[1,2], A. M. HEMMERLE[1], B. A. GARNER[1], O. EKHATOR[3], S. M. FLEMING[1,2,3], K. B. SEROOGY[1,2];
[1]Neurol., [2]Neurosci. Grad. Program, [3]Psychology, Univ. of Cincinnati, Cincinnati, OH

Abstract: Parkinson's disease (PD) is a multifaceted disorder affecting motor movement, emotionality, and cognition. The predominance of the motor deficits, resulting from the degeneration of dopaminergic neurons of the substantia nigra pars compacta, has been the major focus of research efforts. However, degeneration of extranigral brain regions, such as the locus coeruleus (LC) and the dorsal raphe nucleus (DRN), also significantly impact disease pathology. Cell loss in these extranigral areas likely contributes to the non-motor symptoms of PD, including depression, anxiety, cognitive impairment, and olfactory dysfunction. The recent development of new genetic animal models of Parkinson's has allowed for a more in-depth examination of regions outside the nigrostriatal pathway involved in the disease state. In particular, our group has examined the novel DJ-1 knockout (KO) rat. The loss of DJ-1 in humans leads to an autosomal recessive, early onset, familial form of PD. We have previously found that DJ-1 KO rats, in general, exhibit motor hyperactivity compared to wild-type (WT) rats. The goal of this study was to determine the viability of using the novel model to study extranigral areas and their associated behaviors. DJ-1 KO and WT animals were assessed for non-motor deficits via a battery of behavioral tasks, including the elevated plus maze (EPM), novel object recognition task (NOR), and buried pellet test. Animals were then sacrificed, processed for immunohistochemistry, and unbiased stereological cell counts of the LC and DRN were conducted. At 8 and 16.5 months of age, no differences in anxiety-like behavior occurred between DJ-1 KO rats and their WT counterparts in the EPM task. At 15 months of age, DJ-1 KO rats displayed alterations in attention and memory compared to WT animals in the NOR task. At 16 months of age, in the buried pellet test, DJ-1 KO animals demonstrated an increase in

olfactory acuity compared to WT animals. Stereological cell counts of tyrosine hydroxylase-positive (TH+) neurons in the LC showed a reduction of TH+ cells in the DJ-1 KO rats versus WT counterparts. In the DRN, cell counts of tryptophan hydroxylase-positive (TPH+) cells exhibited a strong trend toward a loss of TPH+ cells in DJ-1 KO animals. Overall, DJ-1 KO rats display alterations in non-motor behavior, loss of TH+ cells in the LC, and a trend toward a loss of TPH+ cells in the DRN. While it remains necessary to perform behavioral testing and cell counts on younger DJ-1 KO animals, these results underscore the utility of the DJ-1 KO animals for evaluation of non-motor symptoms and corresponding neurodegeneration in a genetic rat model of PD.

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Poster

676. Parkinson's Disease: Rodent Models II

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Topic: C.03. Parkinson’s Disease

Support: Science and Engineering Research Board, DST, Govt. of India

Title: Admixing of two mice strains with differential susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) positively modulates the nigral dopaminergic phenotype

Authors: *V. D. J, H. YARREIPHANG, T. R. RAJU, P. A. ALLADI;
Neurophysiol., Natl. Inst. of Mental Hlth. and Neuro Scie, Bangalore, India

Abstract: An interesting aspect of Parkinson’s disease (PD) is that it has differential prevalence among different ethnic groups. For example, PD is less prevalent in Asian-Indians compared to Caucasians. Further, the Anglo-Indians who are the admixed population of European and Asian-Indian origin are 5 times lesser susceptible to PD than the latter. We intend to understand this phenomenon of differential prevalence using mice strains, which have differential susceptibility to develop Parkinsonian features upon MPTP administration. We performed systematic neuroanatomical evaluation of dopaminergic neurons of substantia nigra pars compacta (SNpc) in C57BL/6 mice (MPTP susceptible), CD-1 mice (MPTP resistant) and their crossbreds to understand the phenomenon of differential susceptibility to MPTP induced neurotoxicity. The dopaminergic neurons of the SNpc of adult C57BL/6, CD-1 mice and the first filial generation of their reciprocal crosses namely F1X1 and F1X2 were studied at 15-17 weeks (n=6/group). F1X1

were the progeny of female C57BL/6 and male CD-1 mice whereas the F1X2 progeny were that of male C57BL/6 and female CD-1 mice. Unbiased stereology on tyrosine hydroxylase (TH) immunostained serial midbrain sections (40µ) provided the absolute neuronal numbers; densitometry based image analysis and Western blotting revealed protein expression levels; and morphometry revealed their cellular characteristics. Stereological quantification showed significantly higher number of nigral dopaminergic neurons in CD-1 and the crossbred mice compared to C57BL/6. Further, there were insignificant differences in the neuronal densities except for the F1X2 crossbreds. Both, densitometric analysis and immunoblots revealed significantly higher TH expression in the crossbreds compared to C57BL/6. The neuronal nuclear and soma area of the F1X1 and C57BL/6 matched well; while those of FIX2 and CD-1 were similar. The presence of higher number of nigral neurons in CD-1 mice provides the anatomical evidence for its resistance to MPTP induced toxicity. The comparability of numbers in crossbreds and CD-1 mice, complemented by higher TH expression, argue for similar neuroprotection in the crossbreds too. The morphological differences in dopaminergic neurons of susceptible and resistant strains indicate that neuronal size may be a significant factor of vulnerability. Thus, our study provides neuroanatomical evidence of differential susceptibility in mice and the subsequent crossing provides an interesting experimental paradigm to study the human phenomenon of differential prevalence of Parkinson's disease.

Disclosures: V.D. J: None. **H. Yarreiphang:** None. **T.R. Raju:** None. **P.A. Alladi:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation

Title: Intranasal insulin protects against 6-OHDA-induced dopaminergic neuron loss in the substantia nigra and alleviates motor behavioral deficits in rats

Authors: *Y. PANG[1], S. LIN[2], L.-T. TIEN[3], J. SHEN[1], C. WRIGHT[1], L.-W. FAN[1], A. BHATT[1], R. SAVICH[1];
[1]Univ. Mississippi Med. Ctr., Jackson, MS; [2]Univ. of Mississippi Med. Ctr., Jackson, MS; [3]Fu Jen Catholic Univ., New Taipei City, Taiwan

Abstract: Intranasal administration of insulin has been demonstrated to reach brain parenchyma and improve cognitive functions in both animal models and human subjects. Since insulin can activate both insulin and insulin-like growth factor-1 (IGF-1) receptors, which are expressed in the brain and linked to cell survival signaling, we wanted to test whether intranasal insulin could protect against dopaminergic (DA) neuronal damage and motor behavioral impairment in the 6-OHDA neurotoxic rat model of Parkinson's disease. Method: 6-OHDA was injected stereotactically into the right striatum of male adult rats, and the control rats received the same amount of vehicle. Starting from 24 h post 6-OHDA lesion, rats were treated intranasally with recombinant human insulin once a day, until day 14. Neurobehavioral tests were conducted from day 8 to 15 to assess the integrity of nigrostriatal dopamine system. After completion of behavioral tests, brain sections were prepared for stereological estimation of DA neuron numbers in the substantia nigra SN (SN). Results: Exposure to 6-OHDA led to significant motor deficits and a 50% loss of DA neurons (immunostained with tyrosine hydrolase, TH) in the ipsilateral SN pars compacta in rats. Intranasal insulin treatment significantly ameliorated 6-OHDA-induced motor behavioral impairments including locomotor activity ($p<0.05$), tapered/ledged beam walking performance ($P<0.05$), Vibrissa-elicited forelimb-placing ($p<0.05$), initial step ($p<0.05$), and methamphetamine-induced rotational behavior ($P<0.05$). Stereological cell counting showed that intranasal insulin significantly protected against 6-OHDA-induced loss of loss of TH+ DA neurons in the SN pars compacta ($p<0.05$). Intranasal insulin did not affect body weight and blood glucose levels. Conclusion: This study showed that intranasal insulin provides a strong protection against 6-OHDA-induced DA neuronal loss in rats, suggesting that insulin signaling may be a novel therapeutic target in a broad neurodegenerative disorders.

Disclosures: **Y. Pang:** None. **S. Lin:** None. **L. Tien:** None. **J. Shen:** None. **C. Wright:** None. **L. Fan:** None. **A. Bhatt:** None. **R. Savich:** None.

Poster

676. Parkinson's Disease: Rodent Models II

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Thomas Hartman Center for Parkinson's Research at Stony Brook University - Pilot Award (M. Kritzer)

SUNY Brain Network of Excellence Award (W. Collins)

Title: Unilateral nigrostriatal dopamine lesions produce transient changes in urinary and motor function in a rat model of Parkinson's disease

Authors: *W. F. COLLINS, III, O. Y. WANG, N. P. PHAGU, Y. KAMMILI, M. F. KRITZER;
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Abstract: Previous studies utilizing unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway in rats implicate dopamine (DA) loss in the basal ganglia as contributing to the bladder overactivity seen in Parkinson's Disease. The goal of this study is to further characterize this 6-OHDA-lesion model system focusing on the time course of changes induced in lower urinary tract (LUT) and motor function. Under isoflurane anesthesia, adult female Sprague-Dawley rats (225-250 g) received unilateral microinjections of 6-OHDA (2 ul; 4.8 mg/ml) or vehicle into the substantia nigra pars compacta (SNc). Two or four weeks following the injection/lesion, rats were anesthetized with urethane (1.4 g/kg) and set up for cystometry (via bladder dome) and external urethral sphincter (EUS) EMG recording. Following recording, rats were perfused transcardially with 4% paraformaldehyde, and brains were removed and processed for tyrosine hydroxylase immunocytochemistry to confirm lesions. Urine output was measured (overnight in metabolic chamber) and rotation behavior was quantified (open field scoring) every 4-7 days prior to and following the 6-OHDA or sham surgeries and immediately before the cystometry. At two weeks post SNc lesion, increases in voiding frequency were observed both in awake rats (22% decrease in time between voids and 20% increase in number of voids) and during cystometry (46% decrease in threshold volume) compared to sham-operated controls (data are consistent with earlier work (1)). However, at 4 weeks, no difference in bladder function (voiding frequency or void volume) was seen between 6-OHDA-lesioned and sham rats. Further, there was no effect of 6-OHDA lesion on other measures of bladder function (pressure threshold, maximum contraction pressure) or measures of EUS function (duration of EUS bursting, EUS bursting frequency, sustained EUS activity) at either the 2 or 4 week time point. In contrast, 6-OHDA-lesioned rats exhibited strong turning and rotational behavior toward the lesioned side at both 2 and 4 weeks post-lesion. In sum, unilateral nigrostriatal DA depletion in adult female rats has sustained effects on rotational behavior, but only transient effects on LUT function that are limited to increases in voiding frequency. The return of voiding frequency to control levels at the longer survival time suggests that compensatory mechanisms have been activated. Given the medial positions of midbrain, brainstem and spinal circuits that are engaged in LUT function, some sort of sprouting response from the intact side should be considered among candidate mechanisms. 1. Yoshimura et al., (2003). Br J Pharmacol, 139(8), 1425-1432.

Disclosures: W.F. Collins: None. **O.Y. Wang:** None. **N.P. Phagu:** None. **Y. Kammili:** None. **M.F. Kritzer:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.26/D33

Topic: C.03. Parkinson's Disease

Support: Howard Hughes Medical Institute through the Precollege and Undergraduate Science Education Program

New York State Regional Economic Development Council

Binghamton University Provost Office

Title: Cognitive and motor deficits in a rodent model of Parkinson's disease displaying concurrent dopamine and acetylcholine loss

Authors: *C. Y. OSTOCK[1], M. M. CONTI[2], T. LAROSE[1], S. MEADOWS[2], C. BISHOP[2]; [1]Freshman Res. Immersion/ Psychology, [2]Psychology, Binghamton Univ., Binghamton, NY

Abstract: Dopamine (DA) loss in Parkinson's disease (PD) is frequently accompanied by degeneration of acetylcholine neurons within the basal forebrain (BF) and the pedunculopontine nucleus (PPN). Recently, Ach neurons in these nuclei have been implicated in both the motor and non-motor symptoms of PD. However, few rodent models of PD actually account for Ach loss in both the BF and PPN. Here, we evaluated the effects of concurrent BF and PPN Ach loss alone and in combination with striatal DA loss on motor and cognitive performance in a rat model of PD. Sprague-Dawley rats (N = 44) received bilateral: striatal 6-OHDA lesions to deplete DA (DA-lesioned; n = 14), BF (192 IgG-Saporin) and PPN (anti-ChAT Saporin) saporin lesions to deplete Ach (Ach-lesioned; n = 10), combined 6-OHDA + saporin lesions (dual-lesioned; n = 6) , or sham lesions (n = 14). Following recovery from surgery, rats underwent a battery of motor and cognitive behavioral tests. Results indicated that Ach-lesioned and dual-lesioned rats displayed spatial memory deficits on the Morris Water Maze and Spontaneous Alternation tests. DA and Ach lesions alone impaired stepping for the forepaw adjusting steps and vibrissae-elicited paw placement tests and this deficit was exacerbated in dual-lesioned rats. However, only rats with Ach or dual lesions showed motor deficits on the rotarod tests. Collectively, these findings demonstrate that Ach loss may exacerbate cognitive and motor symptoms in PD and highlight the importance of including Ach loss in preclinical models of PD.

Disclosures: C.Y. Ostock: None. **M.M. Conti:** None. **T. LaRose:** None. **S. Meadows:** None. **C. Bishop:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 676.27/D34

Topic: C.03. Parkinson's Disease

Title: Fundamental differences in Parkinsonian rat limbic regions contribute to anxious behavior and diminished responsiveness to diazepam

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Abstract: There is growing recognition that anxiety has a greater impact on the quality of life in Parkinson's disease (PD) than motor symptoms. Yet, little is known about the pathophysiology underlying anxiety present in 25-51% of PD patients, posing a considerable barrier in developing effective treatment strategies. As an important first step, we examine whether anxiety-like behavior is more prevalent in parkinsonian compared to non-parkinsonian rats, mirroring what is observed in the clinic. We used the unilateral, medial forebrain bundle 6-hydroxydopamine (6-OHDA) rat model of PD ('PD rat') and noted that these rats had higher baseline anxiety-like behavior than sham or naïve rats based on their performance in the elevated plus maze (EPM). Next, we administered diazepam, a commonly used acute anxiolytic, prior to EPM, open field test (OFT), and marble burying testing to unmask differences in efficacy between sham and PD rats. We found that diazepam (1.5 mg/kg) loses efficacy in high anxiety PD rats in the EPM and OFT compared to high anxiety sham rats, suggesting differences in neurocircuits involved in anxiety. Lastly, we monitored neuronal spiking activity in the anterior cingulate cortex (ACC), the bed nucleus of the stria terminalis (BNST) and the basolateral amygdala (BLA), all key regions in the limbic circuit, during administration of either yohimbine alone, or diazepam pre-treatment followed by yohimbine in sham and PD animals. Diazepam is able to prevent yohimbine-induced increases in activity in sham rats, but not in PD rats. Altogether, our findings posit that anxiety in PD and non-PD may differ due to differences in neurocircuits in limbic brain areas.

Disclosures: K.A. O'Connor: None. **A. Ramirez-Zamora:** F. Consulting Fees (e.g., advisory boards); TEVA neuroscience. **E. Molho:** 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.; Merz Pharmaceuticals, CHDI, Kyowa Hakko Kirin Pharma, US World Meds, Auspex Pharmaceuticals, Acadia Pharmaceuticals. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); US World Meds. F. Consulting Fees (e.g., advisory boards); US World Meds, Merz Pharmaceuticals, Lundbeck. **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.; Boston Scientific, Medtronic, St. Jude, NIH. F. Consulting Fees (e.g., advisory boards); Boston Scientific, Medtronic, St. Jude. **D. Shin:** None.

Poster

676. Parkinson's Disease: Rodent Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: DA034783

Title: Parkin knockout rats are hypersensitive to the neurotoxic effects of methamphetamine

Authors: *B. A. KILLINGER[1], A. MOSZCZYNSKA[2];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by the progressive loss of nigrostriatal dopamine neurons, which produces symptomatic motor disturbances. Loss-of-function mutations of Park2, a gene encoding the E3 ligase parkin, have been found in patients with familial PD. Despite intense investigation, the exact role of parkin in the development of PD is still unclear. It is not known whether loss of parkin influences dopamine signaling and/or increases the susceptibility of dopamine neurons to environmental neurotoxic insult. Here we tested the hypothesis that parkin knockout (PKO) rats display abnormal dopaminergic neurotransmission, without gross dopamine loss, in the nigrostriatal pathway. To test this hypothesis we administered both toxic (6 mg/kg, 4 injections, 2 h apart, i.p.) and non-toxic (2 mg/kg, 1 injection, i.p.) doses of the potent dopamine agonist methamphetamine (METH) to PKO rats and measured open-field locomotor behavior. We have found that PKO rats develop acute motor behavior abnormalities both during and following binge METH. These motor behavior abnormalities are consistent with those seen in animal PD models and manifest themselves following the third administration of toxic METH. Strikingly, several animals (40%) displayed a complete loss of motor control following the fourth dose of

METH. Although these PKO rats recovered, they continued to display long-term motor deficits, including rigidity, tremors, difficulty initiating movement, and uncoordinated movements. Such abnormalities in motor behavior are consistent with large deficits (>70%) in striatal dopamine. In agreement, we observed significant reductions (-75%) in striatal dopamine content in PKO rats treated with binge METH. We also found that PKO rats display an abnormal locomotor response to a single injection of low-dose METH. In general, low doses of METH transiently increase locomotor activity while high dose METH suppresses locomotor activity. Here, PKO rats displayed the inverse reaction to single injection of METH. Drug naïve PKO rats had a blunted locomotor response to a single low dose of METH while displaying an exacerbated response to a single-high dose METH. Despite the apparent insensitivity of PKO rats to METH-induced hyperlocomotion these animals did not have reductions in striatal dopamine or its metabolites. In summary, we demonstrate that PKO rats are hypersensitive to the neurotoxic effects of METH while conversely being hyposensitive to METH-induced hyperlocomotion. This suggests that dopamine signaling in the nigrostriatal pathway is likely impaired in PKO rats, which may predispose them to neurodegeneration.

Disclosures: B.A. Killinger: None. **A. Moszczynska:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

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Topic: C.03. Parkinson’s Disease

Support: Edwin Brophy Endowment

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MSU Discretionary Funds

Title: Mechanisms of alpha-synuclein mediated toxicity in the aged rat brain

Authors: *I. M. SANDOVAL, N. K. POLINSKI, B. DALEY, N. MARCKINI, F. P. MANFREDSSON, C. E. SORTWELL, T. J. COLLIER;
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Abstract: Parkinson’s disease is a neurodegenerative disorder associated with the abnormal accumulation of alpha-synuclein (α-syn) in protein aggregates, and the subsequent death of the dopaminergic (DA) neurons of the *substantia nigra* (SN). Although aging has been singled out

as the major risk factor for the development of the disease, very little is known about the mechanisms of α-syn mediated toxicity in the context of the aged brain. Previous work from our group determined that significantly less α-syn protein is required to cause equivalent dopaminergic cell death in young and old rats; suggesting a higher vulnerability of the aged nigrostriatal system to α-syn induced toxicity. We hypothesized that equal α-syn overexpression will cause significantly more neurotoxicity in the SN in the brain of aged rats when compared to the young rats. Further, a recent study showed that nuclear pore permeability is compromised in neurons of the aged brain. Therefore, we aim to determine whether age influences the localization and accumulation of α-syn to the nucleus, possibly interfering with normal cellular processes, consequently increasing the vulnerability of DA neurons. To this end, young (2 month) and old (20 month) male Fischer rats received a single intranigral injection of rAAV2/9 expressing human wildtype α-syn (rAAV2/9-α-syn) into the SN. Immunostaining of brain sections collected at 30 days post-injection showed robust and similar α-syn expression in both: young and old rats. Stereological counts of tyrosine hydroxylase immunoreactive (THir) neurons revealed that α-syn overexpression resulted in significantly fewer surviving THir neurons in the SN of aged rats (52%) as compared to young rats (86%). Experiments are underway to elucidate the subcellular localization of α-syn in DA neurons of young and old rAAV2/9-α-syn treated rats, and potential aberrant interaction with nuclear components. Results from these studies will help define aging as a key contributor to neuronal cell death and possibly shed light on the molecular mechanisms underlying the pathophysiology of PD.

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Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 677.02/D37

Topic: C.03. Parkinson’s Disease

Support: MJFF RRIA

Title: Bpoz-2 gene therapy ameliorates alpha-synucleinopathy in a53t transgenic mouse model of Parkinson’s disease

Authors: *A. ROY[1], S. B. RANGASAMY[2], M. KUNDU[1], K. PAHAN[1];
[2]Neurolog. Sci., [1]Rush Univ. Med. Ctr., Chicago, IL

Abstract: Ankyrin-rich BTB/POZ domain containing protein-2 or BPOZ-2 has been recently shown to control ubiquitination of many biological proteins ranging from embryonic development to tumor progression. However, its role in the process of neuronal diseases has not been properly explored. Previously, we reported that BPOZ-2 could play a crucial role in the amelioration of alpha-synuclein (AS) in the cultured dopaminergic (DA) neurons. However, this observation cannot be warranted until its role is tested *in vivo* in the animal model of PD. Here we report that lentiviral administration of bpoz-2 gene indeed lowers the burden of AS in DA neurons in the nigra of A53T transgenic (Tg) mouse. Our detailed immunohistochemical and immunoblot analyses have clearly shown that the overexpression of bpoz-2 dramatically improves both the somatic and neuritic AS pathologies in the nigral DA neurons. Similarly, the specific ablation of bpoz-2 by lentiviral-shRNA fails to ameliorate, but strongly increases the load of monomeric and polymeric forms of AS in the nigral DA neurons of A53T Tg mice as revealed by different immunological analyses. Our results have demonstrated that bpoz-2 gene therapy could be prospective in the amelioration of alpha-synucleinopathy in PD and other Lewy body diseases.

Disclosures: **A. Roy:** 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.; MJ FOX Foundation for Parkinson's Research RRIA. **S.B. Rangasamy:** None. **M. Kundu:** None. **K. Pahan:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson’s Disease

Support: NIH-5P20GM103653

Title: The Ubc9 SUMO-conjugase associates with and regulates α-synuclein SUMOylation, enhancing its half-life and aggregate formation

Authors: *E. CARTIER, H. KIM;
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Abstract: Parkinson’s disease (PD) is the most common motor neurodegenerative disorders characterized by deterioration of the nigrostriatal pathway and loss of dopaminergic neurons in the substantia nigra. Accumulation of toxic aggregates of the synaptic protein α-synuclein is

critical to cause PD pathology. Abnormally oligomerized or aggregated α-synuclein leads to the formation of distinctive Lewy bodies which are the histological hallmark for PD. α-synuclein misfolding and aggregation can be produced by missense mutations and increase in gene dosage both causing familial forms of PD. On the other hand, α-synuclein clearance is determined by degradation through both the proteasome and lysosome systems and its impaired degradation appears to underlie the accumulation of potentially toxic aggregates. In addition, α-synuclein postranslational modifications including ubiquitination, phosphorylation, and nitrosylation have been reported to play a key role in its toxicity. More recently, α-synuclein has been found to be SUMOylated in cultured cells and mouse brain. Small Ubiquitin-like modifier (SUMO) can be targeted to several lysine residues within the molecule, although lysines 96 and 102 have been identified as the main SUMOylation sites. It is still controversialwhether α-synuclein SUMOylation triggers α-synuclein-mediated protein aggregation or it enhances its solubility. Hence, the relationship between SUMOylation and protein solubility/aggregation, and the relevance of SUMOylation to α-synuclein toxicity need to be further investigated. Here, we developed N27 rat dopaminergic immortalized cell lines over-expressing the human E2 SUMO-conjugase enzyme: Ubc9 that increases SUMOylated species, and the Sentrin-specific protease 1 (SENP1), SUMO peptidase that cleaves SUMO from its targets. We found that Ubc9 is able to strongly associate with α-synuclein assessed by co-immunoprecipitation. Moreover, Ubc9 over-expression is able to enhance α-synuclein overall expression by increasing its protein half-life which leads to the accumulation of α-synuclein aggregates. Overall, our data suggest that SUMOylation is a prominent post-translational modification, which extends α-synuclein half-life and its accumulation may lead to potentially toxic aggregates. Our goal of this study is to identify that SUMOylation of α-synuclein can be a potential therapeutic target to modify the PD pathology.

Disclosures: **E. Cartier:** None. **H. Kim:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 677.04/D39

Topic: C.03. Parkinson’s Disease

Support: R01 NS038065

R01 NS086074

Title: Developing novel α-synuclein binding peptides to identify, monitor, and inhibit α-synuclein fibril formation

Authors: *A. R. BRAUN[1], D. R. WOLDRING[2], B. HACKEL[2], M. K. LEE[3];
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Abstract: Parkinson's disease (PD) pathology is characterized by the deposition of insoluble α-synuclein (αS) fibrils. Recent studies, both *in vitro* and *in vivo*, have shown that synthetic αS preformed-fibrils (PFFs) are capable of inducing αS associated pathology; supporting a "prion-like" hypothesis for the spread and progression of PD. In addition to PFFs, on-pathway oligomers, which precede and contribute to αS fibril formation, have been shown to seed fibrilization *in vitro* and exhibit acute cytotoxicity when transfected into cells. Because αS aggregates are thought to be pathogenic in PD, reducing αS aggregation or monitoring the distribution of αS aggregation is considered an important target for disease modifying therapy. Therapeutic targeting of αS aggregates for PD necessitates reliable quantitative biomarkers for αS pathology. Using yeast surface display (YSD) we are engineering a series of novel αS-binding peptides that specifically target αS fibrils and oligomers. Using YSD library based on two different protein scaffolds, fibronectin (14kD) and gp2 (4kD), we identified positive binding clones. The identified clones are being evolved using cycles of mutagenesis and binder screening to obtain peptides with a range of different binding affinities. These positive binding peptides will be evaluated for their capacity to bind, track, and potentially inhibit aggregation of αS in a variety of *in vitro* and *in vivo* models. Strong performing peptides may provide a novel lead for additional modifications for use in future therapy development.

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Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

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Topic: C.03. Parkinson's Disease

Title: Modulation of the microglial inflammatory response to alpha-synuclein oligomers by heparin-induced GAPDH prefibrils

Authors: *J. E. SEPULVEDA DIAZ[1], S. B. SOCIAS[2,1], C. AVILA[2], C. M. TORRES-BUGEAU[2], D. PAPY-GARCIA[3], P. P. MICHEL[1], R. N. CHEHIN[2], R. RAISMAN-VOZARI[1];

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Abstract: In Parkinson's disease (PD) brain, the protein alpha-synuclein (alpha-syn) accumulates to form intra- and extracellular amyloid aggregates called Lewy bodies (LB) and Lewy neurites. The aggregation process occurs progressively through the production of intermediary (e.g., oligomeric) species that form mature and insoluble fibrillary structures. Two sort of arguments suggest that he multifunctional enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) may intervene in the aggregation process: (i) GAPDH colocalizes with α-syn in amyloid deposits from PD brains and (ii) it also promotes the formation of LB-like aggregates in cell culture models. Still consistent with this view, we have shown previously that heparin-induced GAPDH prefibrillar species (HI-GAPDHpf) strongly modify alpha-syn aggregation kinetics and reduce the neurotoxicity of alpha-syn species by accelerating the conversion of toxic oligomers (alpha-syn-oli) into less toxic fibrils (Avila et al, J Biol Chem, 2014). In the present work, we aimed to evaluate the ability of HI-GAPDHpf to modulate brain inflammatory processes in response to alpha-syn-oli (samples prepared according to procedures described by Avila et al, 2014) through the use of a model system of microglial cells in culture. Microglial cells activated by alpha-syn-oli (500 nM; 24h) displayed a hypertrophic morphology and showed increased expression of the ionized calcium-binding adapter molecule 1. Moreover, alpha-syn-oli treatment led to increased production and release of two pro-inflammatory cytokines (TNF-alpha and IL-1beta) and to enhanced generation of intracellular radical oxygen species. We will describe how inflammatory processes are modulated by HI-GAPDHpf in alpha-syn-oli-activated microglial cells.

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Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Title: Lewy Body extracts from Parkinson's disease Brains as models for high-throughput screens of neurotoxicity and α-synuclein spreading from cell-to-cell through endocytosis

Authors: *E. BEZARD[1], F. CAVALIERE[2], L. CERF[3], B. DEHAY[1], M. BOURDENX[1], P. RAMOS-GONZALEZ[2], J. OBESO[4], C. MATUTE[2], F. ICHAS[3];
[1]Inst. of Neurodegenerative Dis., Bordeaux, France; [2]Univ. del Pais Vasco, Bilbao, Spain; [3]Fluofarma, Pessac, France; [4]Ctr. Integral en Neurociencias, Madrid, Spain

Abstract: The anatomo-pathological landmark of Parkinson's disease (PD) is the deposit of aggregated proteins of aberrant conformation into Lewy bodies (LB). Misfolded α-synuclein (α-syn) is a major protein component of LB. Recent data suggest that α-syn can act like a prion-like protein in PD. Recently, through an innovative strategy based on the purification of aggregated α-syn from the Substantia Nigra pars compacta (SNpc) of PD patients, we assessed the prion-like properties of endogenous α-syn assemblies in an elegant ascending series of animal models, ranging from wild-type mice and non-human primates (Recasens et al. Ann. Neurol. 2014). Besides validating the infectious nature of aggregated α-syn, our approach allowed establishing that authentic α-syn assemblies, e.g. LB, possess "pathological" characteristics absent in α-syn assemblies made *in vitro* that account for the specific infectivity of assemblies. Nevertheless, the actual type of neurotoxic insult wreaked upon neurons by α-syn assemblies, the mechanism of passage from cell-to-cell as well as the cellular identity of cells amenable to α-syn assemblies transfer remains unknown. Using human LB-derived α-syn assemblies proven to induce both nigrostriatal damage and spreading all over the brain in wild-type mice, we found that those α-syn assemblies applied to cortical neuron primary culture in 96 well-plates exhibited a modest cytotoxicity but dose-dependently inhibited the growth of neurite length as well as the number of branching points using high-content time-lapse imaging. We then studied the passage from neuron-to-neuron, neuron-to-glia, glia-to-neuron and glia-to-glia in microfluidic experiments. Those experiments demonstrated that human LB-derived α-syn assemblies are uptaken by all cell type by endocytosis and passed from neuron to neuron, from glia to glia, from glia to neuron and from neuron to glia, grounding the mechanism for spreading both in interconnected regions (neuronal transfer) and within large structures (glial cells might cover large territories within a nucleus). In conclusion, we demonstrated the unique behaviour of human LB-derived α-syn assemblies and developed an *in vitro* high-throughput screening platform for identification of potential therapeutics against α-syn assemblies-induced toxicity and spreading.

Disclosures: **E. Bezard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac neuroscience. **F. Cavaliere:** None. **L. Cerf:** A. Employment/Salary (full or part-time):; Fluofarma. **B. Dehay:** None. **M. Bourdenx:** None. **P. Ramos-Gonzalez:** None. **J. Obeso:** None. **C. Matute:** None. **F. Ichas:** A. Employment/Salary (full or part-time):; Fluofarma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Fluofarma.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: Nu Rho Psi

Robert Rich

Title: Alpha-synuclein and heavy metals: Examining the neuromodulatory role of human native alpha-synuclein in cadmium transport dynamics and homeostasis using a dopaminergic cell model of Parkinson's disease

Authors: *W. CHONG[1], G. KWAKYE[2];
[2]Neurosci., [1]Oberlin Col., Oberlin, OH

Abstract: Despite the idiopathic nature of most forms of Parkinson's disease (PD), animal models and postmortem PD tissues exhibit degeneration and concurrent appearance of protein aggregates known as Lewy bodies (LBs). The protein alpha-synuclein (α-syn) comprises 60% of LBs, implicating α-syn in PD neuropathology. While research has revealed mutant α-syn's role in PD, the function of native α-syn, which is expressed throughout the neurotypical brain, remains ill-defined. Here, we examined α-syn's neuromodulation of metal-induced toxicity via a gene-metal screen by utilizing an established dopaminergic N27 cell line that stably expresses α-syn or a vector control (vec). We report that the 10 metals tested (Cd(II), Fe(III), Zn(II), Se, Co(II), Cu(II), Ni(II), Pb(II), Mn(II) and Al(III)) induce varying levels of toxicity in both genotypes. Depending on the metal, toxicity was attenuated or enhanced in α-syn cells relative to vec. We found a novel gene-metal interaction in which 24h cadmium exposure (Cd(II)) causes a concentration-dependent increase in neurotoxicity for α-syn cells compared to vec. Furthermore, we demonstrate that α-syn cells exhibited reduced levels of the antioxidant glutathione and a concomitant increase in intracellular reactive oxygen species (ROS) compared to vec, thus implicating oxidative stress pathways in α-syn-mediated cell death. Expression levels of proteins involved in oxidative stress pathways (including nuclear-factor (erythroid-derived 2)-like 2 (Nrf2) and heme oxygenase 1 (HO-1)) were measured to determine upstream oxidative stress inducers in α-syn - Cd(II) interaction. We observed a decrease in Nrf2 and an increase in HO-1 levels in α-syn cells compared to vec cells after 6h exposure to 100 μM Cd(II). We also hypothesized that α-syn might regulate metal homeostasis via metal transporter neuromodulation and thus we examined the protein expression levels of metal transporters divalent metal transporter 1 (DMT1) and transferrin. Following that, we hypothesize that increased transporter activity will cause greater Cd(II) influx to α-syn cells compared to vec cells. Thus, we sought to

quantify intracellular Cd(II) levels following a 6h and 24 h 110Cd(II) exposure in N27 cells. We utilized inductively coupled plasma mass spectrometry (ICP-MS) to demonstrate that intracellular α-syn cells accumulate more Cd(II) than vec cells, even at non-toxic concentrations (as low as 25nM). Together, these findings have established the role of human native α-syn in heavy metal homeostasis especially Cd transport dynamics. In summary, we have identified a novel gene-environment interaction between Cd exposure and Parkinson's disease.

Disclosures: W. Chong: None. **G. Kwakye:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

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Topic: C.03. Parkinson's Disease

Support: CRC1080

Title: Contrasting homeostatic failure of *in vivo* activity by dopamine substantia nigra neurons in response to aging or mutant alpha-synuclein

Authors: *M. SUBRAMANIAM, J. ROEPER;
Inst. For Neurophysiol., Frankfurt am Main, Germany

Abstract: Dopamine (DA) neurons in the substantia nigra (SN) compacta are selectively vulnerable in Parkinson disease (PD) resulting in progressive neurodegeneration. While multiple stressors such as ageing, environmental toxins (e.g. rotenone, MPTP) and genetic predispositions (e.g. A53T-SNCA) have been identified as key risk factors for PD (Sulzer, 2007), the pathophysiological responses to these stressors by DA SN neurons are less well understood. We have recently shown that DA SN neurons selectively increase their *in vivo* firing frequencies in response to mutant a-synuclein expression or proteasome inhibition (Subramaniam et. al., 2014a/b). In both models, neighboring DA neurons in the ventral tegmental area (VTA) were not affected. In contrast, our recent comparison of *in vivo* firing of DA SN neurons in adult (3-4 month) and aged (22-25 month) C57BL6 mice showed no significant differences in the spontaneous frequency within the two groups. However, the data revealed a significant reduction of median firing frequencies in caudal compared to rostral DA neurons from old mice. As seen in our PD models, only the median frequencies of DA neurons were different within the SN subpopulations (caudal and rostral DA SN neurons) of the old mice, but not their median burstiness or action potential duration. Also, the *in vivo* properties of the DA VTA neurons were

not changed. We also extend our proteasome-inhibition model (Subramaniam et. al., 2014b) to include caudal DA SN neurons. In contrast to the significant increase of *in vivo* firing frequencies in rostral DA SN neurons (Subramaniam et. al., 2014b), DA neurons located caudal SN showed no differences in their median *in vivo* firing frequencies compared to controls although they degenerated more severely (~70% loss of TH positive neurons) in response to expoxomicin. Together our data shows that aging and other PD risk factors do not act in a simply unidirectional fashion on the functional properties of DA SN neurons, but induce an opposed homeostatic activity failure (increase/decrease) with sub-regional specificity. We are currently studying the underlying biophysical mechanisms underlying the frequency changes in the caudal and rostral DA SN neurons.

Disclosures: M. Subramaniam: None. **J. Roeper:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 677.09/D44

Topic: C.03. Parkinson's Disease

Title: Alpha-synuclein affects trace metal content in primary neuronal cultures

Authors: *E. CARBONI[1], P. LINGOR[1], A. CARMONA[2], S. RODEAU[2], E. BARSKI[1], R. ORTEGA[2];
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Abstract: Parkinson's disease (PD) is the most frequent neurodegenerative movement disorder worldwide and it affects about 1% of the population over 65 years of age. The protein alpha-synuclein (aSyn) is the major component of proteinaceous inclusions, so-called Lewy bodies (LB), which are the hallmark of the disease. Interestingly, there is also an increase of iron in the LB and there is evidence for a disturbed metal homeostasis in PD patients' brains, but these mechanisms are still poorly understood. In this study we asked, whether aSyn itself could affect the distribution of trace metals in primary neurons and if aSyn regulates the expression of proteins involved in metal transport. To this, aSyn was overexpressed in primary cell cultures of rat midbrain neurons and the medium was supplemented with iron to mimic the metal dyshomeostasis of PD patients' brains. Cultures were then subjected to highly sensitive particle-induced X-ray emission (PIXE) imaging allowing for spatial localization of a given element within the cell and the quantification of its absolute concentration. Cells overexpressing aSyn

showed higher concentrations of intracellular calcium and iron after iron supplementation compared to controls. In the absence of iron supplementation, cells overexpressing aSyn showed significantly higher levels of copper compared to control. We then characterized the regulation of proteins involved in the cellular transport of trace elements, such as the Divalent Metal Transporter 1 (DMT1), the Copper Transporter 1 (Ctr1) and the Transferrin Receptor (TfR). Our data suggest that aSyn can influence the expression levels of metal transport proteins, like the copper importer Ctr1. In conclusion, our study shows that the increased levels of aSyn alter the intracellular concentrations of transition metals and the expression patterns of metal transport proteins. Transition metals and/or it's transport proteins can thus be promising therapeutic targets for the treatment of Parkinson's disease.

Disclosures: E. Carboni: None. **P. Lingor:** None. **A. Carmona:** None. **S. Rodeau:** None. **E. Barski:** None. **R. Ortega:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 677.10/D45

Topic: C.03. Parkinson's Disease

Title: Modulation of synaptoneurosome glutamate release by aggregated proteoforms of α-synuclein

Authors: *J. B. WATSON[1], K. LITTLEJOHN[1], S. YUAN[1], B.-K. KOO[2], J. P. WHITELEGGE[1], T. A. SARAFIAN[1];
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Abstract: α-Synuclein, a central protein involved in Parkinson's disease (PD), exists *in vivo* in a variety of modified and aggregated forms associated with progressive PD pathology. However, the specific proteoform structures involved with neuropathological disease mechanisms are not clearly defined. Since α-synuclein is known to play a role in presynaptic vesicle dynamics, we developed an *in vitro* assay to measure glutamate neurotransmitter release using mouse brain synaptoneurosomes (SNs). Using this approach, we observed previously that transgenic mice overexpressing human α-synuclein did not display detectable fibril or oligomer formation but had enhanced SN glutamate release relative to wildtype (WT) controls [T Sarafian et al, 2013 PLoS ONE 8(5): 1-15. doi: 10.1371/journal.pone.0063557; JB Watson et al, 2014, Soc Neurosci Abstr 413.230]. To examine more systematically the presynaptic role of aggregated proteoforms,

fibrils were first prepared *in vitro* by prolonged agitation of recombinant human α-synuclein in a Fluoroskan plate-reader in the presence of either NaCl or GdnHCl and analyzed by fluorescence (TFT, DCVJ, ANS probes), Native/SDS-PAGE, and electron microscopy. Relative to a histone control and monomeric α-synuclein, functional experiments show that pre-incorporation of fibrillated forms of human α-synuclein enhanced potassium/calcium-stimulated glutamate release from WT mouse SNs. Experiments in progress will address the ability of the more physiologically relevant, amino-terminally acetylated α-synuclein to form oligomers and fibrils and to modulate SN glutamate release. Overall results suggest that fibrillated forms of α-synuclein increase glutamate neurotransmitter release and may cause excitotoxicity as one component of their neuropathology in PD.

Disclosures: J.B. Watson: None. **K. Littlejohn:** None. **S. Yuan:** None. **B. Koo:** None. **J.P. Whitelegge:** None. **T.A. Sarafian:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 677.11/D46

Topic: C.03. Parkinson’s Disease

Title: The oxysterol 27-hydroxycholesterol-induced epigenetic regulation of α-synuclein

Authors: *O. GHRIBI, J. SCHOMMER;
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Abstract: Accumulation of the α-synuclein (α-Syn) protein in Lewy body inclusions is a hallmark of synucleinopathies. Despite extensive research, no disease-modifying therapy is currently available forsynucleinopathies and the search for diagnostic tests and biomarkers are still under development. The search for disease-modifying therapies or diagnostic markers would benefit from identification of factors that promote over-production of α-Syn protein and elucidation of new cellular mechanisms that regulate the transcription of α-Syn. The role of α-Syn in the pathogenesis of synucleinopathies is not understood, but experimental studies point to a potential neurotoxic role of high levels of this protein in either its soluble or aggregated form. The causes of synucleinopathies are likely multi-factorial with genetic susceptibility and non-genetic factors potentially participating in the increase in the expression levels of α-Syn. While genetic mutations are responsible for about 5 to 10% of all the forms of Parkinson’s disease, the most prevalent synucleinopathy, the vast majority of the cases are sporadic, with epigenetic modifications potentially playing a role in the α-Syn accumulation. Of high relevance to the

regulation of α-Syn expression levels are our findings showing that the cholesterol metabolite27-hydroxycholesterol (27-OHC) increases α-Syn expression levels *in vitro*. While epigenetic modifications, such as DNA methylation, alter α-Syn transcription, the extent to which 27-OHC regulates α-Syn through methylation is yet to be determined. α-Syn gene (SNCA) has two CpG islands, one located in the first axon and the second in the first intron. We determined the methylation status of CpG1 and 2 using Bisulfite Sequencing method in SH-SY5Y cells treated with various concentrations of 27-OHC. Bisulfite specific PCR based sequencing demonstrated that the CpG-2 sequences are methylated under vehicle treatments and the methylation dose-dependently decreases with 27-OHC treatments. Bisulfite analysis showed no changes in CpG-1 methylation with addition of 27-OHC. We also found that increased α-Syn levels is not associated with cellular stress. The causes of accumulation of α-Syn in synucleinopathies are not known.Our results may help in understanding aspects of the cellular mechanisms involved in the pathogenesis of diseases related to α-Syn accumulation.

Disclosures: **O. Ghribi:** None. **J. Schommer:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

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OSOTF CRND Graduate Student Aid Endowment

Title: Identification of protein interactions regulated by alpha-synuclein Serine 129 phosphorylation

Authors: M. M. MARANO, K.-C. HAN, M. S. FRASER, T. F. LANGMAN, *A. TANDON; Tanz Ctr. for Res. in Neurodegenerative Dis., Univ. of Toronto, Toronto, ON, Canada

Abstract: The objective of this study is to identify and characterize novel protein interactions with alpha-synuclein (α-syn), the central protein involved in neurodegenerative disorders broadly classified as synucleinopathies (Parkinson’s disease, multiple systems atrophy, Dementia with Lewy bodies). In these diseases, α-syn aggregates into cytoplasmic inclusions located in neurons

or glial cells, ultimately resulting in cell death. Over 90% of aggregated α-syn in protein inclusions is phosphorylated at the serine 129 position, while less than 10% of α-syn is phosphorylated in healthy controls. It is believed this phosphorylation is involved in the pathogenicity of α-syn. To assess the effects of α-syn phosphorylation, an α-syn interaction screen was performed using homogenates from whole brain or brain synaptosomes obtained from B6129x1-Snca tmlRos1/J α-syn knockout mice. These lysates were eluted through columns containing agarose beads cross-linked to either non-phosphorylated or phosphorylated wild-type human α-syn that had been phosphorylated by polo-like kinase 2, which phosphorylates α-syn at the Ser129 position. Proteins bound specifically to the column were eluted and then analyzed by mass spectrometry to yield a list of potential interacting proteins. These findings have important significance, as they identify novel protein interactions that are differentially regulated by α-syn phosphorylation. Using these preliminary results, candidate proteins were selected from this list and will be further investigated. Interactions will be confirmed using reciprocal co-immunoprecipitation and the interactions will be characterized by assessing for co-localization with α-syn. Following these initial studies this project will be extended to more focused work relating to the candidate protein to examine how phospho-Ser129 α-syn may regulate its function. The increased prevalence of phospho-Ser129 α-syn in disease pathology suggests it is related to protein aggregation, although it remains unclear if it is involved in the initiation of α-syn aggregation or merely a downstream effect. Examining proteins that interact with phospho-Ser129 α-syn may provide clues as to the role of α-syn phosphorylation in normal and pathological conditions, potentially leading to novel therapeutic targets.

Disclosures: M.M. Marano: None. **K. Han:** None. **M.S. Fraser:** None. **T.F. Langman:** None. **A. Tandon:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 677.13/D48

Topic: C.03. Parkinson’s Disease

Support: Project funding is from a private donation

Title: Secretion and uptake of alpha-synuclein via extracellular vesicles in cultured cells

Authors: *C. I. LÖÖV[1], L. BALAJ[1], J. BERGSTROM[2], X. O. BREAKEFIELD[1], B. T. HYMAN[1], M. INGELSSON[1,2];

[1]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]Publ. Hlth. and Caring Sci., Uppsala Univ., Uppsala, Sweden

Abstract: Background Observations both *in vivo* and on cell culture indicate that propagation of pathology in the Parkinson's disease brain may be caused by cell to cell spreading of α-synuclein. The underlying mechanisms are largely unknown, although recent evidence suggests that exosomes or other extracellular vesicles may be involved. Here, we investigated release and uptake of different α-synuclein species via vesicle-related mechanisms. **Objective** To assess, in cultured cells, whether different forms of α-synuclein can be secreted and taken up via extracellular vesicles (EVs). **Methods** The human neuroblastoma SH-SY5Y cell line, cultured in media with vesicle-depleted serum, was either non-transfected or transfected with non-tagged α-synuclein or α-synuclein:hemi-YFP. Ultracentrifugation at 100,000x*g* generated EV-fractions and supernatants, which were measured for total α-synuclein levels by ELISA. Western blot and Nanosight tracking analysis were used to assess the presence of vesicles in the respective fractions. To study α-synuclein uptake, non-transfected SH-SY5Y cells were incubated with the EVs or supernatants from transfected and non-transfected cells or with recombinant α-synuclein. **Results** Nano- to picomolar levels of α-synuclein could be detected in cell lysates and in the secreted fractions from transfected cells. Transfection with α-synuclein:hemi-YFP displayed a significantly higher ratio of secreted vs retained protein compared to cells transfected with untagged α-synuclein. Moreover, the α-synuclein:hemi-YFP transfected cells displayed a higher proportion of α-synuclein in the EV fraction than in the supernatant compared to cells transfected with non-tagged α-synuclein. However, only a small amount of the extracellular α-synuclein was internalized independent of source. Western blot and Nanosight tracking analysis indicated that the vesicular fractions were enriched for flotilin-1 positive exosomes. **Conclusions** Our data suggest that human neuroblastoma cells secrete α-synuclein both as free-floating protein and via vesicles that are characteristic of exosomes. The α-synuclein:hemi-YFP fusion protein are secreted to a larger extent than untagged α-synuclein and seem to be particularly directed towards vesicular secretion. Finally, cellular uptake of α-synuclein in this model is modest and does not seem to differ between proteins that have been released from transfected cells or that have a recombinant origin.

Disclosures: C.I. Lööv: None. **L. Balaj:** None. **J. Bergstrom:** None. **X.O. Breakefield:** None. **B.T. Hyman:** None. **M. Ingelsson:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

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Program#/Poster#: 677.14/E1

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS057656

Title: α-Synuclein disrupts the intracellular trafficking of iron import proteins (Fet3, Ftr1) in Saccharomyces cerevisiae

Authors: *S. N. WITT, D. PATEL;
LSU Hlth. Sci. Ctr. / Biochem., Shreveport, LA

Abstract: The main culprits in Parkinson's disease (PD) are elevated levels of both the neuronal protein α-synuclein (α-syn) and iron. Our hypothesis is that elevated levels of α-syn disrupt the normal intracellular trafficking of iron export proteins to the plasma membrane; instead, in cells with α-syn, the iron transporters divert to the lysosome for degradation. Aberrant trafficking of the iron export proteins leads to iron accumulation and cell death. We have been studying how α-syn disrupts iron homeostasis in yeast cells, and because yeast and humans have similar proteins that mediate iron uptake/export, our results will likely be relevant to humans. In humans, ceruloplasmin (Cp), which is a soluble ferrooxidase that mediates iron export from cells, has been genetically linked to PD. Another human protein, hephaestin, which is a membrane-bound ferroxidase, also mediates iron export. Yeast express Fet3, which is an ortholog of Cp and Hp. Fet3 with its binding partner Ftr1 mediate iron import into yeast cells. A soluble form of Fet3 fully complements for the loss of Cp in aceruloplasminemic mice (Cp-/- mice), and Hp complements for the loss of Fet3 in budding yeast. The experiments described here sought to determine whether human α-syn disrupts the intracellular trafficking of Fet3-Ftr1 complexes in yeast. We found that α-syn dramatically shortens the life span of non-dividing, stationary-phase yeast cells. For example, the median lifetime (t1/2) was 13.5 ± 5 d and 4.0 ± 1 d without and with α-syn expression, respectively. Probing the mechanism underlying this accelerated cell death, we discovered that the stationary-phase cells expressing α-syn have approximately 30% less iron than the same cells without α-syn expression, and the level of Fet3 was signficiantly lower in cells expressing α-syn than in contreol cells without α-syn. These results show that low iron may be the cause of the shortened lifespan of cells expressing α-syn. To further test this hypothesis, WT cells were grown in medium supplemented with iron (10 μM FeCl3) and then we performed the aging assay (±α-syn). Supplemental iron partially rescued accelerated aging of those cells expressing α-syn; specifically t1/2 increased from 4.0 d to 7.8 d. Lastly, we also co-expressed mouse Hp and human α-syn in yeast cells and found that α-syn decreases the level of Hp. These results indicate that α-syn disrupts the intracellular trafficking of Fet3/Hp, which causes iron deficiency and cell death. Experiments are underway to test whether α-syn drives Hp to the lysosome in human cells.

Disclosures: **S.N. Witt:** None. **D. Patel:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Lundbeck Foundation

Danish Council of Independent Research

Title: Activated Microglia Modulate α-synuclein secretion via JNK activation in α-synucleinopathic PC12 catecholaminergic neurons

Authors: *D. P. CHRISTENSEN[1,3], P. EJLERSKOV[4], I. RASMUSSEN[2], F. VILHARDT[2]; [1]Univ. of Copenhagen, Cambridge, MA; [2]Univ. of Copenhagen, Copenhagen, Denmark; [3]Dept. of Cell. and Mol. Med., Copenhagen, Denmark; [4]Biotech Res. & Innovation Ctr., Copenhagen, Denmark

Abstract: Although α-SNC lacks a secretory signal it can be released both as mono- and oligomeric forms by unconventional secretion pathways during cellular stress, possibly to be taken up by neighboring cells (neurons or glia). Microglia (the resident brain macrophages) are essential for PD development and we hypothesize that they play an hitherto unappreciated role in PD pathogenesis. In PD numerous activated microglia have been found in, but not limited to, the vicinity of degenerating neurons. Upon activation microglia can induce severe neurotoxic effects by excess production of cytotoxic factors such as superoxide and tumor necrosis factor alpha (TNFα). Recently, we have established a cell culture system modeling PD with NGF-differentiated PC12 neurons conditionally expressing the Lewy body associated protein tubulin polymerization-promoting protein (TPPP/p25α) and α-SNC (wt or A30P). In this culture model α-SNC is secreted by the exocytosis of α-SNC containing autophagosomes and amphisomes (exophagy). We investigated mediators of neuronal α-SNC secretion and if neurons and microglia interact to influence neuronal α-SNC secretion. Pharmacologic (SP600125) and genetic (shRNA) JNK knockdown decreases, whereas constitutive active JNK signaling (fusion construct transfections) increases neuronal α-SNC release (assessed by protein precipitation and immunoblotting of conditioned media). Lipopolysaccharide (LPS) activated inflammatory microglia increase neuronal α-SNC release in co-culture and TNFα (classically derived from activated microglia) exposure of neurons in monoculture mimics the effect of activated inflammatory microglia. Thus activated microglia may not only inflict bystander damage to neighboring neurons, but could also mediate proteopathic spreading of α-SNC from neuron to

neuron.Collectively, our data support a critical role for activated inflammatory microglia in the proposed hypothesis for PD spreading throughout the brain.

Disclosures: D.P. Christensen: None. **P. Ejlerskov:** None. **I. Rasmussen:** None. **F. Vilhardt:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson’s Disease

Support: NS088206

NS078247

Title: Fyn kinase mediates aggregated α-synuclein import and priming of the NLRP3 inflammasome in microglia

Authors: *N. PANICKER, S. SARKAR, M. NEAL, D. HARISCHANDRA, H. JIN, H. SAMINATHAN, V. ANANTHARAM, A. KANTHASAMY, A. KANTHASAMY; Biomed. Sci., Iowa State Univ., Ames, IA

Abstract: Persistent neuroinflammation is recognized as a key pathophysiological contributor to many neurodegenerative diseases, including Parkinson’s disease (PD). Resident brain microglia mediate chronic neuroinflammation through the production of pro-inflammatory factors. Identifying the key molecular signaling events perpetuating microglial activation could unravel novel mechanisms that contribute to progressive neurodegeneration in PD. The NLRP3 inflammasome, traditionally shown to be involved in the innate immune response to microbial pathogens and particulate entities, has recently been implicated in neurodegenerative diseases. However, its role in PD pathogenesis is yet to be established. Herein, we show that aggregated human α-synuclein, the major component of Lewy bodies, can activate the inflammasome pathway in microglia. Aggregated α-synuclein treatment amplified LPS-induced priming of the NLRP3 inflammasome in mouse primary microglia, synergistically promoting NLRP3 and pro-IL-1β induction, as well as subsequent IL-1β processing and caspase-1 activation, culminating in the secretion of cleaved IL-1β into the supernatant. This inflammasome activation was accompanied by significantly increased nitrite production and NOS2 expression. Pre-treatment of the cells with pan caspase or caspase-1 specific inhibitors significantly blocked aggregated α-

synuclein-induced IL-1β production in primary microglia. We then demonstrate that aggregated α-synuclein can mediate both priming and maturation of the NLRP3 inflammasome independent of LPS stimulation. Next, we examined the role that the non-receptor Src family tyrosine kinase Fyn plays in aggregated α-synuclein-mediated inflammasome activation. Fyn was found to be rapidly activated in microglial cells by aggregated α-synuclein and it contributed to NF-κB activation, which induced pro-IL-1β and NLRP3 message levels. Strikingly, Fyn was also found to play a role in the import of α-synuclein into microglial cells. Finally, we observed diminished production of IL-1β and other pro-inflammatory cytokines from Fyn-deficient microglia in response to aggregated α-synuclein stimulation. Collectively, our results demonstrate that the NLRP3 inflammasome plays a role in the inflammatory response during α-synuclein and other protein misfolding and that Fyn may regulate NLRP3 inflammasome-signaling in PD. (Supported by NS078247 and NS088206).

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Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: 1R21NS088923-01

Rapid Response Innovation Awards, 2014 awarded by Michael J Fox Foundation

Title: Pum2-mediated translational regulation of alpha-synuclein mRNA in neurites and crosstalk with mitochondria

Authors: ***Y.-S. KIM**[1], G. JE[2], S. GUHATHAKURTA[3], S. BASU[3], E. BOK[3], A. CRISTOVAO[4];
[1]Biomed. Sci., Burnett Sch. of Biomed. Sci., Orlando, FL; [2]Col. of Med., Kyung-Hee Univ., SEOUL, Korea, Republic of; [3]Burnett Sch. of Biomed. Sci., Univ. of Central Florida, Orlando, FL; [4]CICS-UBI Hlth. Sci. Res. Ctr., Univ. of Beira Interior, Covilhã, Portugal

Abstract: Alpha-synuclein (-SYN) is a central molecule in the pathogenesis of Parkinson’s disease (PD). We have recently found that regulation of α-SYN could occur at mRNA level in

the 3'UTR de-pendent manner. Human α-SYN transcripts have relatively long 3'-untranslated regions (3'-UTRs) compared to other species and transcripts having various composition of the 3'-UTR have been reported. However, the precise contribution of the 3'-UTRs to healthy physiology and pathological processes in PD is largely unknown. Here, we show that α-SYN mRNA is translocated into neurites of fully differentiated human dopaminergic neuronal cells, supporting its physiological role in neurites. RNA-binding protein Pum2 is responsible for neuritic localization and translational repression by recognizing a conserved binding motif in the α-SYN 3'-UTR. Mi-tochondrial reactive oxygen species relieve Pum2-mediated translational repression, increasing α-SYN level in neurites. Interestingly, α-SYN mRNA is highly enriched in mitochondria and pro-motes local protein translation, increasing mitochondrial oxygen consumption and ATP generation. Collectively, Pum2-dependent translational control of α-SYN near mitochondria contributes to the fine-tuning of mitochondrial respiratory functions in neurites. Dysfunction of these mechanisms may contribute to PD pathogenesis.

Disclosures: Y. Kim: None. **G. Je:** None. **S. Guhathakurta:** None. **S. Basu:** None. **E. Bok:** None. **A. Cristovao:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

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Program#/Poster#: 677.18/E5

Topic: C.03. Parkinson's Disease

Support: 1R21NS088923-01

Title: De-repression of TET1 by oxidative stress regulates alpha-synuclein expression in dopaminergic neurons

Authors: *S. GUHATHAKURTA[1], S. BASU[2], E. BOK[2], G. JE[3], Y.-S. KIM[2];
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Abstract: In human, the gene (SNCA) coding for alpha-synuclein (α-SYN) contains high CpG rich region around transcription start site encompassing promoter and intron1. Hypomethylation of this intron1 CpG island has been shown to be associated with deregulated higher expression of α-SYN in Parkinson's disease (PD). However, this finding remains controversial across different studies and also the detailed mechanism behind this regulation has not been explored. SNCA being a high CpG containing gene is bound by active histone modification H3K4me3 which

favors transcription. Interestingly, we found that in matured dopaminergic neuronal cell line, SNCA expression level is significantly less as compared to non-neuronal cells such as HEK-293T. To understand the underlying reason of this less expression of SNCA in dopaminergic neuronal cell line, we found that this gene is also co-occupied by histone repression modification H3K27me3. This peculiar presence of both the activation and repression marks together made this gene promoter “bivalent” in the neuron which supports its lesser expression. Based on this gene's epigenetic structure i.e. presence of CpG island and promoter bivalency, we hypothesized that SNCA could be regulated by Ten Eleven Translocase 1 (TET1). Expectedly, we observed that TET1 binds to SNCA promoter and knocking down of TET1 leads to huge expression of α-SYN whereas the basal expression of α-SYN further goes down upon overexpression of the cysteine rich DNA binding CXXC domain of TET1. This finding demonstrated that TET1 acts as a major repressor for α-SYN in dopaminergic neurons. It is already known that α-SYN can be regulated by Reactive Oxygen Species or ROS. To understand how ROS can regulate this gene's expression, we checked the SNCA promoter driven luciferase activity in presence of TET1-CXXC domain with or without known ROS inducers like Paraquat and H2O2. We found that the repression of SNCA promoter driven luciferase activity by TET1-CXXC is reversed in presence of oxidative stress. In order to understand how this de-repression of TET1 or release of its binding to SNCA happens, we measured the cysteine oxidation status of this protein. Since, TET1 is a cysteine rich protein and 8 of them are involved in its DNA binding, there is a high chance that these residues could get oxidized to its sulphenylated forms. Interestingly, we found that TET1 cysteine sulphenylation is increased in presence of ROS which might be the cause for this de-repression of TET1 from the SNCA gene promoter and allow its transcription.

Disclosures: **S. Guhathakurta:** None. **S. Basu:** None. **E. Bok:** None. **G. Je:** None. **Y. Kim:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

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Program#/Poster#: 677.19/E6

Topic: C.03. Parkinson’s Disease

Support: 1R21NS088923-01

Title: Implication of 8-oxodG-mediated Transcriptional Mutagenesis in sporadic Parkinson’s disease

Authors: *S. BASU[1], S. GUHATHAKURTA[2], E. BOK[3], G. JE[4], A. CRISTOVAO[5], Y.-S. KIM[2];
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Abstract: Oxidative stress-mediated DNA damage in the form of 8-oxo-7,8-dihydroguanine (8-oxodG, oxidized guanine), has been shown to accumulate under several disease like Cancer and neurodegeneration including Parkinson's disease (PD). In post-mitotic cells like neurons, where fidelity of transcription and translation is key to maintaining physiological activity, 8-oxodG-mediated misincorporation of adenine instead of cytosine in mRNA chain during transcription (transcriptional mutagenesis, TM) can lead to significant phenotypical and functional alterations. Sporadic PD is classically characterized by aggregation of a synaptic protein called alpha-synuclein (α-SYN), which forms intraneuronal inclusion in dopaminergic neurons. Genomic mutations that can alter the conformation of α-SYN to β-sheet structure are known to accelerate the aggregation. We hypothesized using SNCA (alpha-synuclein) gene as a model, that TM can generate novel variants at the mRNA level which can accelerate the aggregation process. We predicted the generation of 43 possible mutants of α-SYN through Transcriptional Mutagenesis (TM), but focused on a few which had the highest potential for making the molecule more prone towards aggregation based on prediction by a structural analysis algorithm, TANGO. We confirmed the presence of two of the predicted mutations (Serine42Tyrosine(S42Y) and Alanine53Glutamate(A53E)) in SNCA mRNA from the substantia nigra region of human post-mortem PD brain using RNaseH2 based PCR technique. S42Y and A53E mutations when present accelerate oligomerization of α-SYN compared to wild-type protein using protein complementation system. To our knowledge, this is the first report showing the presence of TM related mutations in Parkinsonian tissue and their role in the pathogenesis of the disease.

Disclosures: S. Basu: None. **S. Guhathakurta:** None. **E. Bok:** None. **G. Je:** None. **A. Cristovao:** None. **Y. Kim:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 677.20/E7

Topic: C.03. Parkinson's Disease

Support: The National Basic Research Program of China (2011CB504102 and 2012CB722407)

National Natural Science Foundation of China (81371398)

Natural Science Foundation of China (7131001)

Title: Alpha-synuclein overexpression negatively regulates insulin receptor substrate 1 by activating mTORC1/S6K1 signaling

Authors: *H. YANG;
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Abstract: Alpha-synuclein (α-Syn) is a major component of Lewy bodies, a pathological feature of Parkinson's and other neurodegenerative diseases collectively known as synucleinopathies. Among the possible mechanisms of α-Syn-mediated neurotoxicity is interference with cytoprotective pathways such as insulin signaling. Insulin receptor substrate (IRS)-1 is a docking protein linking IRs to downstream signaling pathways such as phosphatidylinositol 3-kinase/Akt and mammalian target of rapamycin (mTOR)/ribosomal protein S6 kinase (S6K)1; the latter exerts negative feedback control on insulin signaling, which is impaired in Alzheimer's disease. Our previous study found that α-Syn overexpression can inhibit protein phosphatase (PP)2A activity, which is involved in the protective mechanism of insulin signaling. In this study, we found an increase in IRS-1 phosphorylation at Ser636 and downregulation of tyrosine phosphorylation, which accelerated IRS-1 turnover and reduced insulin-Akt signaling in α-Syn-overexpressing SK-N-SH cells and transgenic mice. The mTOR complex (C)1/S6K1 blocker rapamycin prevented the phosphorylation of IRS-1 at Ser636 in cells overexpressing α-Syn, suggesting that mTORC1/S6K1 activation by α-Syn causes feedback inhibition of insulin signaling via suppression of IRS-1 function. α-Syn overexpression also inhibited PP2A activity, while the PP2A agonist C2 ceramide reversed both S6K1 activation and IRS-1 Ser636 phosphorylation upon α-Syn overexpression. Thus, α-Syn overexpression negatively regulated IRS-1 via mTORC1/S6K1 signaling while activation of PP2A reverses this process. These results provide evidence for a link between α-Syn and IRS-1 that may represent a novel mechanism for α-Syn-associated pathogenesis.

Disclosures: H. Yang: None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 677.21/E8

Topic: C.03. Parkinson's Disease

Support: The Swedish Research Counsil´s International post doc fellowship

The Sweden-America Foundation Post Doc Fellowship

Title: Heparan sulfate is involved in cellular uptake of alpha-synuclein amyloid fibrils but not oligomers

Authors: *E. IHSE[1], J. D. ESKO[2], E. MASLIAH[3];
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Abstract: Objectives: Spreading of the pathology to increasingly larger areas of the brain in neurodegenerative diseases like Alzheimer´s and Parkinson´s disease has been proposed to be caused by a prion-like seeding mechanism. It has been shown that protein aggregates involved in these diseases can transfer from one cell to another, but not much is known about how the secretion or internalization occurs. The glycosaminoglycan heparan sulfate is known to interact with protein aggregates that are having the amyloid fold, as it has been found in protein deposits of essentially every amyloid disease regardless of the aggregating protein. It has also recently been shown that heparan sulfate is involved in the cellular uptake of A-beta, tau and prion protein aggregates. The objective of the present project was to investigate if heparan sulfate are involved in cellular internalization of alpha-synuclein aggregates, and if this is true only for aggregates with an amyloid fibril conformation or also for oligomeric species. Methods: Alpha-synuclein in oligomeric or fibrillar conformation was added to the cell media of rat neuroblastoma B103 cells or CHO-cells deficient in heparan sulfate all together or in N- or 2-O-sulfation of the heparan sulfate chains. Cellular uptake was determined by ELISA on cell lysates. Colocalization of heparan sulfate and alpha-synuclein aggregates was studied through confocal microscopy. Results: Cellular uptake of fibrillar alpha-synuclein was almost completely abolished by the addition of heparin to the cell media of B103 cells, while uptake of oligomers were only slightly inhibited. In the microscope, both oligomeric and fibrillar alpha-synuclein were seen to colocalize with heparan sulfate in the B103 cells, in what seems to be endocytic vesicles. All of the CHO mutants showed strongly inhibited uptake of fibrillar alpha-synuclein compared to wt cells, but there was no significant difference in uptake ability between the different mutants. In contrast, no significant difference of uptake was seen between the CHO wt and mutant cells for oligomeric alpha-synuclein. Conclusions: Heparan sulfate is involved in the cellular uptake of some, but not all, types of alpha-synuclein aggregates, and the amyloid fibrillar conformation is likely important for this interaction.

Disclosures: **E. Ihse:** None. **J.D. Esko:** None. **E. Masliah:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS089622

National Parkinson Foundation

Title: Inclusion seeding by mutant α-synuclein fibrils in primary neuronal cultures and in transgenic mice

Authors: *N. J. RUTHERFORD, M. BROOKS, B. I. GIASSON;
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Abstract: α-synuclein (αS) is a major player in the pathogenesis of Parkinson's disease (PD) as the protein is found to be aggregated within Lewy body inclusions in the brains of PD patients. Additionally, missense mutations in, or increased copy number of the gene that encodes αS (SNCA) have been identified in PD families. Some of these missense mutations have been shown to accelerate aggregation *in vitro*, however this is not always the case. Interestingly, two recently identified mutations, G51D and A53E, were reported to slow down the rate at which αS forms fibrils, but increased cellular toxicity under mitochondrial stress conditions and, for A53E, reduced aggregation in cell culture. Previous studies have shown that αS fibrils produced from the PD-causing A53T and E46K αS mutant proteins can induce morphologically distinct inclusions in primary neuronal-glial cultures overexpressing αS, producing flame-like and rounded inclusions respectively. Furthermore, this phenomenon is mirrored in mice transgenic for A53T or E46K αS. It has also been reported that the species of αS fibril, whether wild-type, A53T or E46K, is the determining factor as to which type of inclusion is formed. Therefore our ongoing studies are designed to further investigate the effect of treating αS overexpressing primary neuronal-glial cultures with fibrils produced from all of the identified αS mutants. Quantification of the induction of inclusion pathology and morphological analyses of inclusions will provide information on the abilities of the αS mutants to induce αS inclusion pathology in this cell culture model. In addition, the intramuscular injection seeding followed by CNS transmission model is being utilized in αS transgenic mice, allowing us to assess the physiological relevance of these findings as well as determine the effects of the mutant αS fibrils on toxicity by examining the survival of motor neurons within the lumbar spine. These studies will help to define the inclusion driving capabilities and toxic nature of the different αS mutations in a disease relevant model, providing potential clues to the pathogenesis of this devastating disease.

Disclosures: **N.J. Rutherford:** None. **M. Brooks:** None. **B.I. Giasson:** None.

Poster

677. Alpha-Synuclein Mechanisms in Parkinson's Disease

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 677.23/E10

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation for Parkinson's Research Rapid Response Innovation Award 2013 (EC)

ARCS Foundation (WPF)

Title: Genetic and size determinants of alpha-synuclein mediated vesicle rupture

Authors: W. P. FLAVIN[1], O. I. ZHURBICH[2], S. SKARPATHIOTIS[3], *E. CAMPBELL[4]; [1]Integrative Cell Biol., [2]Microbiology and Immunol., [3]Stritch Sch. of Med., [4]Cell Biology, Neurobio. and Anat., Loyola Univ. Chicago, Maywood, IL

Abstract: Numerous recent studies have increasingly implicated smaller aggregates of alpha-synuclein (a-syn) as the pathological cause of Parkinson’s disease. It is now becoming appreciated that a-syn can spread from cell to cell in a prion-like fashion, propagating its misfolded, aggregated conformation from affected to neighboring cells in much the same way as a spreading infection. Our lab previously demonstrated that a-syn aggregates disruptively enter cells by inducing rupture of lysosomal membranes following endocytosis, causing cathepsin-mediated oxidative stress and inflammasome activation. In order to further investigate the ability of a-syn to induce vesicle rupture, and to test the hypothesis that familial missense mutations can differentially affect the efficacy of a-syn entry in this manner, we generated aggregates of purified WT and mutant forms of a-syn, characterized the aggregate size distribution of these preparations, and measured the ability of these species to induce vesicle rupture. We determined that amine-reactive FITC fluorophore labeling of aggregated a-syn preparations increases the proportion of smaller, single a-syn fibrils in the sample compared to larger, grouped fibrils, and that these smaller, labeled aggregates are able to significantly induce vesicle rupture regardless of a-syn type. Additionally, we demonstrated that cells exposed to insult by different types of a-syn exhibit rupture of distinct intracellular compartments, and that aggregates of missense mutant G51D a-syn demonstrate a significantly increased propensity for inducing rupture of larger vesicles. This work provides further mechanistic insight into the movement of a-syn within and between cells, as well as the detrimental consequences of this spread for affected neurons. Understanding the cellular and molecular mechanisms responsible for intercellular

transfer of a-syn pathology is critical for developing treatments designed to arrest or prevent Parkinson's disease progression.

Disclosures: W.P. Flavin: None. **O.I. Zhurbich:** None. **S. Skarpathiotis:** None. **E. Campbell:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.01/E11

Topic: C.03. Parkinson's Disease

Title: Neuroprotective effects of zonisamide against lactacystin-induced neurodegeneration do not involve changes in system xc- expression

Authors: *E. BENTEA[1], J. VAN LIEFFERINGE[1], T. DEMUYSER[1], S. KOBAYASHI[2], L. DENEYER[1], G. ALBERTINI[1], E. MERCKX[1], K. MAES[1], H. SATO[3], I. SMOLDERS[1], J. LEWERENZ[4], A. MASSIE[1];
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Abstract: Zonisamide (ZNS), an anti-epileptic drug used in the symptomatic treatment of Parkinson's disease (PD), has been recently linked with neuroprotective properties in toxin- and genetic-based models of PD. One of the mechanisms proposed to mediate the neuroprotective effects of ZNS involve an increase in expression of the cystine/glutamate antiporter system xc-, leading to enhanced cystine supply for astrocytic glutathione synthesis (Asanuma et al. Ann Neurol 2010 67(2):239-49). At the same time, however, enhancement of system xc- might trigger excitotoxicity due to pathological astrocytic glutamate release, leading to non-cell autonomous neuronal death (Massie et al. FASEB J 2011 25(4):1359-69). In order to gain further insights into the neuroprotective properties of ZNS and elucidate the role of system xc-, we have employed the lactacystin (proteasome inhibition) mouse model of PD. Our findings indicate that chronic treatment with ZNS (30mg/kg; i.p.) protects against lactacystin-induced neurodegeneration, confirming the neuroprotective properties of ZNS, for the first time in a model based on proteasome inhibition. The neuroprotective effects of ZNS were accompanied by an improvement in sensorimotor function, as evaluated using the adhesive removal test. ZNS treatment failed, however, to modulate the expression of system xc- in midbrain and striatum of

lactacystin treated mice, indicating that the neuroprotective action of ZNS do not involve changes in system xc- expression. Similarly, we found that chronic treatment with ZNS did not influence system xc- expression or glutathione levels in the basal ganglia of control (untreated) mice. Finally, *in vitro* studies indicated that ZNS treatment did not change system xc- activity in HT22 cells or primary astrocytes, and did not influence glutathione levels in astroglial C6 cells. In conclusion, our study revealed neuroprotective and symptomatic effects of chronic ZNS treatment in a PD model based on proteasome inhibition. Our top-down approach investigating the effect of ZNS treatment in pathological and physiological conditions and in cell culture, failed to reveal any significant effect on system xc- expression or activity. We thereby propose that the neuroprotective actions of ZNS are not mediated via system xc-. Instead, other pathways are likely to be involved, such as the proposed anti-inflammatory, anti-oxidant and neurotrophic properties of ZNS. Future studies in this regard will be important in understanding the mechanisms of neuroprotection of ZNS. Nevertheless, our findings support the use of ZNS as a symptomatic and possible disease-modifying therapy in PD.

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Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.02/E12

Topic: C.03. Parkinson’s Disease

Support: NIH-5P20GM103653

Title: A novel neuroprotective aurimmed compound as a potential therapeutic for Parkinson’s disease

Authors: *J. E. CAVINESS;
Delaware State Univ., Dover, DE

Abstract: Parkinson’s disease (PD) is the second most prominent neurodegenerative disorder to date affecting over 1% of the U.S. population over the age of 65. PD pathology is characterized by protein aggregation known as Lewy bodies and the depletion of dopaminergic neurons in the substantia nigra (SN). Currently, most commonly used therapeutics focus on alleviating PD symptoms, however, there is no known effective therapeutic that shows neuroprotective or

recovery effects for PD patients. Using cell viability and toxicity (MTT and LDH) assays, we have identified that the lead AurimMed compound: AMP-X-0079 showed a significant neuroprotective and recovery effect on oxidative stress induced toxicity (MPP+ and H_2O_2) in rat dopaminergic N27 cells. In addition to our preliminary *in vitro* studies, we found that AMP-X-0079 increased the survival rate as well as the mobility rate in the rotenone induced *Drosophila* PD model. Furthermore, we assessed the effect of oral AMP-X-0079 treatment in the MPTP-lesioned C57/Bl6 mouse model. Our behavioral data suggest that the oral treatment of AMP-X-0079 (50 µg/gram body weight) for two weeks was sufficient to improve their movement from MPTP-induced damage. In multiple behavioral studies including hindlimb clasping, cross-beam and grooming tests, we found that the compound treated mice showed significantly improved movement, compared with the MPTP and vehicle-treated mice. The following immunohistochemical analysis of the striatum and the SN verified that there is a significant increase in the tyrosine hydroxylase-positive cells in both the striatum and the SN from AMP-X-0079 treated animals when compared to MPTP/Vehicle treated animals. Although we may need to identify the optimal dose for the treatment, the oral treatment of the novel compound already displayed a promising efficacy in mice. We are currently validating over 10 identified target genes in the mechanism of action from mass spectrometry-based protein profile, compared with the vehicle group. We believe that there is a great potential to develop the novel AurimMed compound as an effective therapeutic, not only because it alleviates the PD symptoms and slows the disease progression, but also because it shows neuroprotective/recovery effects in PD models.

Disclosures: J.E. Caviness: None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson’s Disease

Support: France Parkinson

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Carnot Institut

Title: The neuroprotective effects of near infrared light (670nm) when applied at different periods in relation to MPTP insult

Authors: *F. REINHART[1,2], N. EL MASSRI[3], N. TORRES-MARTINEZ[1,2], F. DARLOT[1,2], C. CHABROL[1,2], D. M. JOHNSTONE[3], J. STONE[3], J. MITROFANIS[3], A.-L. BENABID[1,2], C. MORO[1,2];
[1]CEA, Grenoble, France; [2]Univ. Grenoble Alpes, Grenoble, France; [3]Dept of Anat. F13, Univ. of Sydney, Sydney, Australia

Abstract: Parkinson's disease (PD) is the second most widespread neurodegenerative disease around the world. It is characterised by the signs of resting tremor, bradykinesia and rigidity. The standard treatments for PD are effective at attenuating the motor signs at least at their onset. However they do not efficiently slow the progression of the PD. In another hand, a growing number of studies illustrate the interest of near-infrared (NIr) treatment to preserve the motor capacities and to protect the dopaminergic cells in animal models of the PD. We assess here if a NIr therapy could be used to prevent the PD and/or stays efficient even when the disease is well established. We used an acute mouse MPTP model. Briefly, the mice received 2 injections in 2 days (50mg/kg global dose). They also received (or not) a 90s light (670 nm) treatment, extra-cerebrally, twice a day, within 2 days. According to the various groups, the mice received the light treatment either the 2 days before the MPTP injections (pre-treated), or during the MPTP injections (simultaneously treated), or the 2 days after the MPTP injections (post-treated). The motor capacities were assessed in an open field daily. After the MPTP days, the mice were allowed to survive during one week, then a histological analysis have been realised to determine the number of dopaminergic cells preserved. Behaviour: the MPTP group has a 60% decrease of its motor capacities after the MPTP injections and remains very sick the days after. We show here that a pre-treatment preserves the motor capacities as well as a simultaneous treatment, during the entire MPTP time (50% better). The days after the MPTP injections, these two groups recovered their complete motor capacities. In the post-treated group, the mice were very affected before the treatment (60% loss). Only 20min after the light application, this group recovered its complete motor capacities. Histology: the pre- and simultaneous treatment showed a higher number of dopaminergic cells preserved (≈100%) than the MPTP group (≈70%). However, if the post-treatment has a higher number of cells preserved than MPTP (≈ 20% better), this result does not reach the significant limit. We show here that, (1) a 670 nm pre-treatment is protective against the MPTP insult and, (2) a post-treatment improves the motor capacities of parkinsonian mice. To conclude, we can suppose two distinct mechanisms of action of NIr light. (1) A short term effect (a few minutes) leading to motor capacities improvement; (2) a longer term effect, maintained at least 48h, which preserves cells form death. These results confirm the interest of a NIr therapy against the disease and allow us to seriously consider this strategy in human.

Disclosures: F. Reinhart: None. **N. El Massri:** None. **N. Torres-Martinez:** None. **F. Darlot:** None. **C. Chabrol:** None. **D.M. Johnstone:** None. **J. Stone:** None. **J. Mitrofanis:** None. **A. Benabid:** None. **C. Moro:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.04/E14

Topic: C.03. Parkinson's Disease

Support: NS 047198

Title: Development of a novel brain penetrant multifunctional iron chelator dopamine agonist for symptomatic and neuroprotective therapy of Parkinson's disease

Authors: *A. K. DUTTA, Dr[1], B. DAS[1], L. XU[1], T. ANTONIO[2], M. REITH[2];
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Abstract: Parkinson's disease (PD) is a major neurodegenerative disorder affecting 1-2% of the elderly population, causing profound motor impairments that include tremors at rest, rigidity, bradykinesia, and postural instability along with non-motoric symptoms such as autonomic, cognitive and psychiatric problems. It is believed that environmental factors converging on oxidative stress, nigral iron elevation, mitochondrial dysfunction, inflammation and aberrant protein aggregation, account for most cases of PD. For the successful treatment of multifactorial CNS diseases, like PD, a new paradigm has arisen to address the underlying pathogenesis pathways, according to which multifunctional drugs having multiple pharmacological activities can be employed to target more than one pathological factor to slow the disease progression and alleviate motor dysfunction at the same time. Unfortunately, no neuroprotective drugs have been identified or approved by the FDA so far for the treatment of PD, thereby, necessitating research for such agents. To address this yet unmet medical need and considering the implication of free iron (II) in PD, we have designed, synthesized and evaluated a novel hybrid iron chelator, D-607, as a multitarget-directed ligand against PD. In the GTPγS functional assay, the compound revealed full agonist activity at both D2 and D3 receptors (EC50 (GTPγS); D2 = 51.6 and D3 = 13.5 nM). *In vitro*, the molecule displayed potent antioxidant and efficient iron chelation properties along with preferential affinity for the reactive form of the metal (Fe2+) that induces oxidative stress in PD brain. In reserpinized PD animal model, D-607 also exhibited potent *in vivo* activity in reversing hypolocomotion delineating its blood brain barrier crossing ability. Pre-treatment with D-607 *in vitro* was found to rescue dopaminergic PC12 cells from toxicity induced by both 6-hydroxydopamine and iron administration in a dose-dependent manner, thereby, producing neuroprotection effect. These observations strongly suggest that compound D-607 might be an excellent multifunctional agent for a novel viable therapy of PD. This work is supported by grants from NINDS (NS 047198, AKD).

Disclosures: **A.K. Dutta:** None. **B. Das:** None. **L. Xu:** None. **T. Antonio:** None. **M. reith:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.05/E15

Topic: C.03. Parkinson's Disease

Title: Chronic caffeine mitigates aberrant motor learning: Insights into reduced Parkinson's disease risk in caffeine drinking populations

Authors: *A. C. KROK[1], J. L. KORANDA[1], J. A. BEELER[2], X. ZHUANG[1];
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Abstract: Epidemiological studies have consistently shown that heavy caffeine consumption reduces the risk of Parkinson's disease (PD); however, the mechanism of such protective effects remains unknown. In recent studies, we provide evidence that dopamine (DA) blockade can induce motor inhibition through corticostriatal LTP in the indirect pathway. We have proposed that aberrant plasticity (i.e. aberrant learning) may degrade established motor skills and contribute to PD motor symptoms. Additionally, we have shown that the xanthine theophylline, a non-selective adenosine receptor antagonist similar to caffeine, can mitigate both aberrant motor learning and underlying abnormal corticostriatal plasticity. In the current study, we predicted chronic caffeine exposure could also mitigate aberrant motor learning due to its A2A antagonist activity. To test the effects of chronic caffeine on aberrant motor learning, mice were pretreated with caffeine in their drinking water for 2 weeks and then trained on the accelerating rotorod. We found chronic caffeine exposure partially rescued rotorod performance during an initial acquisition phase of training in which DA receptors were pharmacologically blocked. We then retrained the same mice on the rotorod in the absence of dopamine blockade (i.e. recovery phase). Performance of mice withdrawn from caffeine, but not those maintained on caffeine, recovered to levels of control mice that were previously trained with saline rather than dopamine blockade. This result is consistent with an earlier study that showed theophylline diminishes initial acquisition of aberrant motor learning, but theophylline administered after aberrant learning actually impairs recovery. We also found that chronic caffeine reduced the sensitization of haloperidol-induced catalepsy, reinforcing the idea that chronic caffeine may mitigate D2R blockade-induced impairments in motor learning. These results suggest that chronic caffeine exposure may protect against the acquisition of aberrant motor learning, providing protection

against aberrant motor learning that may delay the onset and severity of PD motor symptoms in caffeine drinking populations.

Disclosures: **A.C. Krok:** None. **J.L. Koranda:** None. **J.A. Beeler:** None. **X. Zhuang:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.06/E16

Topic: C.03. Parkinson's Disease

Title: Pre- and post-synaptic mechanisms for nicotine's protective effect against Parkinson's disease

Authors: ***J. KORANDA**[1], A. C. KROK[1], D. S. MCGEHEE[2], J. A. BEELER[3], X. ZHUANG[1]; [1]Neurobio., [2]Anesthesia, The Univ. of Chicago, Chicago, IL; [3]Queens Col. CUNY, Queens, NY

Abstract: Nicotine use has long been associated with decreased risk of Parkinson's disease (PD), but the potential protective mechanisms are unknown. Recently, we reported that dopamine (DA) D2 receptor (D2R) blockade induces an experience-dependent learned inhibition of movement and suggested that this 'aberrant motor learning' contributes to PD symptoms. We have found that chronic nicotine treatment can reduce aberrant motor learning in mice, suggesting that chronic nicotine may exert a PD protective effect through reducing aberrant learning and aberrant corticostriatal plasticity. In the current study, we found that the specific deletion of β2 nicotinic subunits in DA neurons is sufficient to reduce stimulated DA release in anesthetized mice and mitigate aberrant motor learning, similar to chronic nicotine treatment. We also found that chronic nicotine treatment and β2 deletion reduce the phosphorylation of extracellular regulated kinase (pERK) following repeated D2R antagonism paired with the motor learning paradigm. These results suggest that the striatal network may become adapted to non-pathologically low DA levels following chronic nicotine treatment. These neuroadaptations, in turn, may protect against dopamine denervation-induced aberrant motor learning. The current data suggest one possible mechanism that might underlie the protective effects of chronic nicotine against PD.

Disclosures: **J. Koranda:** None. **A.C. Krok:** None. **D.S. McGehee:** None. **J.A. Beeler:** None. **X. Zhuang:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.07/E17

Topic: C.03. Parkinson's Disease

Title: Modeling Parkinson's disease in aged rats: the neuroprotective effects of caffeine against the neurotoxicity of 6-hydroxydopamine

Authors: *G. H. BEAGLEY[1], C. O. HALEY[2];
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Abstract: Parkinson's disease (PD) involves the degeneration of dopaminergic neurons in the substantia nigra (SN). Researchers have hypothesized that the caffeine in coffee is neuroprotective, and is associated with the prevention and treatment of PD. This study investigated whether caffeinated coffee and/or caffeine tablets reduce behavioral and/or cognitive impairment of PD. Two year old male Sprague Dawley rats were stereotaxically injected with 6 - hydroxydopamine (6 - OHDA) producing bilateral lesions in the SN; generating an animal model of PD. Rats were treated with caffeinated coffee and caffeine tablets for at least 2 weeks before surgery and continued on their assigned regimen after surgery. Rats were then tested for cognitive and motor impairment using the Morris Water Maze (MWM). Results indicate, on average, that the rats treated with caffeine were faster at locating the platform than the rats treated with coffee (tablets (1:30 seconds vs 1:53 seconds) and both were faster than rats with no caffeine treatment (2:00 seconds). In addition, behavioral observations indicate that coffee may be better at reducing motor dysfunction. Histological results suggest that caffeinated coffee was better than caffeine tablets at increasing neuronal viability in the substantia nigra in comparison to no treatment. Other bioactive properties of coffee, independent of caffeine, are suspected to play a role in neuroprotection and the treatment of PD.

Disclosures: G.H. Beagley: None. **C.O. Haley:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

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Program#/Poster#: 678.08/E18

Topic: C.03. Parkinson's Disease

Title: Glycyrrhizic acid attenuates dopaminergic neurodegeneration in rotenone model of Parkinson's disease

Authors: ***E. HAQUE**[1,2], H. JAVED[2], S. ABUL KHAIR[2], S. AZIMULLAH[3], S. OJHA[3]; [2]Dept. of Biochem., [3]Dept. of Pharmacol. and Therapeut., [1]UAE Univ., Al Ain, United Arab Emirates

Abstract: Parkinson disease (PD) is the second most common neurodegenerative disorder characterized by loss of dopaminergic neurons in the SNc area. Numerous studies suggest that oxidative stress and inflammation play a critical role in the etiopathogenesis of PD. The current study was undertaken to assess the neuroprotective potential of glycyrrhizic acid (GA), one of the active components present in licorice against rotenone-induced rat model of PD. Since, PD is a progressive chronic neurodegenerative disorder; we have used chronic protocol for four weeks at a dose of 50 mg/kg prior to rotenone (3 mg/kg) challenge. The administration of rotenone caused significant reduction in antioxidant activities as evidenced by reduced activity of superoxide dismutase, catalase and depletion of glutathione with a concomitant rise in the lipid peroxidation product, malondialdehyde. A significant enhancement in the levels of pro-inflammatory cytokines and elevation in the inflammatory mediators like cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) was observed in the midbrain region. The immunohistochemistry revealed that following rotenone challenge, a significant increase in the expression of ionized calcium binding adaptor molecule-1 (Iba-1), and glial fibrillary acidic protein (GFAP), which are indicative of microglial and astrocyte activation accompanied by loss of dopamine neurons in the SNc. Interestingly, GA treatment significantly protected the dopamine neuron and normalized the Iba-1 and GFAP activation from the rotenone insult. GA also improved antioxidant enzymes, prevented glutathione depletion and inhibited lipid peroxidation along with attenuation of induction of pro-inflammatory cytokines. Subsequently, GA also attenuated the increased levels of inflammatory mediators such as COX-2 and iNOS. Based on the results of our study we conclude that GA protects against rotenone-induced PD and the neuroprotective effects are attributed to its potent antioxidant and anti-inflammatory properties.

Disclosures: E. Haque: None. **H. Javed:** None. **S. Abul Khair:** None. **S. Azimullah:** None. **S. Ojha:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.09/E19

Topic: C.03. Parkinson's Disease

Support: AICTE, India

Title: The effect of flavonoids against paraquat induced oxidative stress and neurotoxicity in *Drosophila* melanogaster

Authors: *D. R. JHONSA, L. BADGUJAR, B. SUTARIYA, M. SARAF;
Pharmacol., Bombay Col. of Pharm., Mumbai, India

Abstract: Parkinson's disease is a chronic, progressive neurodegenerative disorder with a multifactorial etiology involving advancing age, environmental and genetic factors. The exact cause of PD is unknown as well as its cure; dopamine (in the form of levodopa or mucuna pruriens) being the gold standard of PD treatment since the discovery of the disorder. But it only provides symptomatic relief from the disease and is unsuccessful in halting the progression of the disease. *Drosophila* melanogaster has widely been used as a model to study a number of neurodegenerative diseases. Exposure to environmental toxin paraquat is employed to induce oxidative stress induced neurotoxicity and sporadic Parkinson's disease. Flavonoids are an important class of phenolic phytochemicals exerting a multiplicity of neuroprotective actions within the brain. In this study, the therapeutic anti-oxidant properties of two flavonoids, fisetin and hesperidin against paraquat induced oxidative damage and neurotoxicity in *Drosophila* melanogaster has been examined. Adult female and male flies (CsBz strain) exposed to 7 and 12 mM paraquat solution for 12 hours showed significant mortality and locomotor disability. The life span was extended and the locomotor activity of flies was enhanced in paraquat exposed flies when treated with fisetin and hesperidin for 24 hours. Fisetin and hesperidin treatment also ameliorated oxidative stress factors such as intracellular ROS levels, superoxide dismutase and catalase. They also reduced acetylcholine esterase activity which was increased by paraquat. Also, the levels of the endogenous anti-oxidant, reduced glutathione which were diminished by paraquat were also enhanced by fisetin and hesperidin. Thus, this study demonstrated that feeding flies with fisetin and hesperidin after exposure to paraquat showed anti-oxidant and neural protective effects leading to recovery of locomotor behaviour and extension of life span in fruitflies. The project also confirmed the utility of *Drosophila* melanogaster as a model in screening putative therapeutic molecules prior to their use in mammalian models.

Disclosures: D.R. Jhonsa: None. **L. Badgujar:** None. **B. Sutariya:** None. **M. Saraf:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.10/E20

Topic: C.03. Parkinson's Disease

Support: National Research Foundation of Korea(NRF) Grant (No. 2014R1A1A2056508)

National Research Foundation of Korea(NRF) Grant (No. 2014R1A1A4A01007858)

Title: Effects of naringin treatment on the neuroprotection and neurorestoration in animal models of Parkinson's disease

Authors: H. KIM[1,2,3], M. JEON[1,2,3], H. JANG[1,2,3], S. KIM[1,2,3], J. PARK[1,2,3], U. JUNG[4], *S. KIM[1,2,3,5];
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Abstract: We recently reported that treatment with naringin, a major flavonoid found in grapefruit and citrus fruits, attenuated neurodegeneration in a rat model of Parkinson's disease (PD) *in vivo*. In order to investigate whether its effects are universally applied to a different model of PD, and whether its treatment induces restorative effects on the lesioned nigrostriatal dopaminergic (DA) projection, we observed the effects of pre-treatment or post-treatment with naringin in a mouse model of PD. For neuroprotective effects, 6-hydroxydopamine (6-OHDA) was unilaterally injected into the striatum of mouse brains for a neurotoxin model of PD in the presence or absence of naringin by daily intraperitoneal injection. Our results showed that naringin protected the nigrostriatal DA projection from 6-OHDA-induced neurotoxicity. Moreover, similar to the effects in rat brains, this treatment induced the activation of mammalian target of rapamycin complex 1 (mTORC1), which is well known as an important survival factor for DA neurons, and inhibited microglial activation in the substantia nigra (SN) of 6-OHDA-treated mice. However, there was no significant change of DA phenotypes in the SN and striatum post-treated with naringin compared with 6-OHDA-lesioned mice, despite the treatment being continued for 12 weeks. These results suggest that post-treatment with naringin alone may not be enough to restore the nigrostriatal DA projection in a mouse model of PD. However, our results apparently suggest that naringin is a beneficial natural product to prevent DA degeneration, which is involved in PD. Corresponding authors: Sang Ryong Kim, PhD and Un Ju Jung, PhD This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (No. 2014R1A1A2056508 and 2014R1A1A4A01007858).

Disclosures: **H. Kim:** None. **M. Jeon:** None. **H. Jang:** None. **S. Kim:** None. **J. Park:** None. **U. Jung:** None. **S. Kim:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.11/E21

Topic: C.03. Parkinson’s Disease

Title: Protective effects of pyrroloquinoline quinone against the cell death caused by 6-hydroxydopamine and hydrogen peroxide

Authors: *Y. YAMADA, M. NAKAMICHI;
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Abstract: Parkinson's disease (PD) is one of most common neurodegenerative diseases with progressive neurodegeneration of the nigrostriatal pathway. Reactive oxygen species (ROS) has been implicated in the etiology of PD. 6-Hydroxydopamine (6-OHDA) is a neurotoxin that acts specifically on nerve cells, are taken up by dopaminergic neurons. It is thought that ROS and endoplasmic reticulum stress (ERS) involved in toxicity of 6-OHDA. Pyrroloquinoline quinone (PQQ) is reported to participate in a range of biological function such as antioxidant and cell growth-promoting effect. It was reported that PQQ rich feed increased mice liver mitochondria. However, the mechanism of these actions is not known in detail. On the other hand, PQQ which has a very high reactivity and readily reacts with the amino group-containing substance has been reported to change imidazol pyrroloquinoline (IPQ) by forming an imidazole skeleton. In this study, we compared the protective effect of PQQ2Na (oxidized form), PQQH2 (reduced form), IPQNa (oxidized form) and IPQ (reduced form) against 6-OHDA and H2O2 using human neuroblastoma cell line SK-N-SH. The cells incubated in the presence of 6-OHDA for 24 hrs with the four forms of PQQ in CO2 incubator or H2O2 for 10 min at room temperature. After incubation, the cell viability rates were measured by MTT assay. In addition, the effects of PQQ2Na, PQQH2, IPQNa and IPQ against mitochondria formation were examined. The expression levels of human cytochrome c oxidase subunit IV isoform 1 (COXIV-1) that is known as one of mitochondria marker genes were measured by RT-PCR using Taq Man probes. All PQQs used in this experiment showed protective effects against the toxicity of 6-OHDA and H2O2. PQQ2Na, PQQH2, IPQNa and IPQ show a trend of protection at concentrations as low as 1 nM and 100 nM against 6-OHDA and H2O2, respectively. PQQ and amino acid adduct IPQ showed the almost same biological activity. PQQ might act as the adduct form *in vivo*. PQQ2Na, PQQH, IPQ and IPQNa increased the expression of COXIV-1 in SK-N-SH. These results suggest that PQQ may play the fundamental role in metabolism of the neuron and will be useful for the development of therapeutic agents of PD.

Disclosures: **Y. Yamada:** None. **M. Nakamichi:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

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Topic: C.03. Parkinson's Disease

Support: BK21 Grant 22A20130012283

NRF-2013R1A1A4A01005837

Title: Multi-therapeutic potentials of silibinin for the treatment and prevention of Parkinson's disease

Authors: ***Y. LEE**;
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease, and induced by a selective loss of dopaminergic neurons in the nigrostriatal pathway. The lipophile MPTP can cross the blood-brain barrier, and is subsequently metabolized into toxic MPP+, which causes mitochondrial dysfunction and the selective cell death of dopaminergic neurons, and thus causes PD pathologies. The flavonoid silibinin is the major active constituent of silymarin, an extract of the milk thistle seeds and known to have hepatoprotective, anti-cancer, anti-oxidative effects and neuroprotective effects. In the present study, we reported the neuroprotective effects of silibinin in both acute and sub-chronic MPTP model of PD. Pre- and post-treatments of silibinin were effective to attenuate motor deficit and dopaminergic neuronal loss caused by acute and sub-chronic PD model, respectively. Glial activation was observed in striatum and in substantia nigra of mice treated with both MPTP-induced PD models. Silibinin had modulatory roles on neuroinflammation in acute PD mice model, but not in sub-chronic PD mice model. In addition, *in vitro* study confirmed that silibinin suppressed MPP+-induced astroglial activation and ERK phosphorylation in primary astrocytes, and also prevented MPP+-induced cell death and disruption on mitochondrial membrane potential in primary cultured neurons. Taken together, the present study indicates that silibinin has multi-therapeutic potentials for treatment and prevention of PD and other neurodegenerative diseases associated with mitochondrial dysfunction or neuroinflammation.

Disclosures: **Y. Lee:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

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Topic: C.03. Parkinson’s Disease

Support: This research has been supported by Grant Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education (#2014R1A1A401007348)

Title: Neuroprotective effects of antidepressants via upregulation of neurotrophic factors

Authors: *S. SHADFAR, D.-Y. CHOI;
Yeungnam Univ., Gyeongsan, Korea, Republic of

Abstract: Neurotrophic factors are essential for neuronal survival, plasticity and development, and have been implicated in the mechanism of the action of antidepressant drugs (ADs). In an attempt to investigate the effects of three different antidepressant drugs on expression of neurotrophic factors and their related signaling pathways, we injected C57Bl6/J mice with ADs including fluoxetine, imipramine and milnacipran (i.p., 20 mg/kg/day for 1 week or 3 weeks). We also measured concentration of monoamines (dopamine, 3,4-dihydroxyphenylacetic acid, serotonin, homovanillic acid) in the striatum of mice after injection of ADs. Our results revealed that all of the drugs upregulated the concentration of brain-derived neurotrophic factor in the striatum both at one week and three weeks after treatment. In contrast, an increase in glial-derived neurotrophic factor was more obvious at three weeks after the antidepressants treatment. Specifically, fluoxetine and imipramine more clearly increased expression of neurotrophic factors than milnacipran. In addition, we found that the ADs raised the phosphorylation of extracellular signal-regulated-protein kinase (Phospho-Erk1/2) and the serine/threonine kinase Akt protein. Moreover, ADs slightly increased monoamine neurotransmitters in the striatum. Therefore, we carefully propose that ADs might have neuroprotective properties and could be employed as novel therapeutic agents for neurodegenerative diseases such as Parkinson’s disease and Alzheimer’s disease.

Disclosures: S. Shadfar: None. **D. Choi:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

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Topic: C.03. Parkinson's Disease

Support: BK21 Grant 22A20130012283

NRF-2013R1A2A2A01067388

Title: Neuroprotective effects of DNP on the MPTP-induced Parkinson's disease mouse model

Authors: *A. KIM;
Pusan Natl. Univ., Pusan-City, Korea, Republic of

Abstract: Parkinson's disease (PD) is progressive neurodegenerative disorder characterized by loss of dopaminergic neurons in substantia nigra (SN) of the brain. 2.4-Dinitrophenol (DNP) is well known for a mitochondrial uncoupler which inhibits efficient energy production in mitochondria. Mounting evidences suggest the beneficial effects of DNP on neural plasticity and neurodegeneration, thus the present study tested effects of DNP on the MPTP-induced PD mouse model. Mice were pretreated with DNP (1 mg/kg or 5 mg/kg) for thirteen consecutive days. Then four times of MPTP (20 mg/kg) was administered into mice to introduce PD pathologies. MPTP caused significant motor deficits and DA neuronal loss in SN of PD mice. However, DNP pretreatment ameliorated MPTP-induced motor dysfunction and dopaminergic neuronal loss in striatum (STR) and substantia nigra (SN). Moreover, we observed that DNP prevented the MPP+-induced cell death and reduction of mitochondrial membrane potentials in SH-SY5Y cells and primary cultured neurons. The present study showed that DNP-mediated mild inhibition of oxidative phosphorylation can be neuroprotective on the MPTP-induced PD mouse model. Current findings suggest that DNP could protect dopaminergic neurons and maintain the integrity of mitochondrial membrane potential in PD through activating adaptive stress responses.

Disclosures: A. Kim: None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

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Topic: C.03. Parkinson's Disease

Support: BK21 Grant 22A20130012283

NRF Grant 2009-0083538

Title: Pretreatment of morin alleviates neurotoxicity in Parkinson's disease mouse model

Authors: *J. LEE;
Pusan Natl. Univ., Busan, Korea, Republic of

Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative disorders and progressive neurodegenerative diseases, and characterized by loss of dopaminergic (DA) neurons in substantia nigra (SN). Morin (3,5,7,2',4'-pentahydroxyflavone), a member of flavonols, is a constituent of many herbs, fruits and wine. Previous studies have shown that morin has antioxidant, anti-inflammatory, and antiproliferative effect *in vivo* models of cancer. In addition its neuroprotective effects are also well reported in neurodegenerative diseases model including Alzheimer's disease. Here we reported the neuroprotective effects of morin in the MPTP-induced PD mouse model. Mice were pre-treated with morin (5mg/kg or 50mg/kg) for twelve consecutive days. Then four times MPTP (20 mg/kg) was given to mice to introduce PD pathologies. MPTP induced significant motor deficits and DA neuronal loss in SN of PD mice. Morin attenuated motor dysfunction induced the PD model, and tyrosine hydroxylase (TH) immunostaining showed that morin protected DA neuronal loss from MPTP-induced PD model in substantia nigra (SN) and striatum (STR). Morin also alleviated MPTP-mediated neuroinflammation in SN and STR. Furthermore, we found that morin pretreatment protected neurons against toxic MPP(+)-mediated cell death in primary cultured neurons. The present study provides *in vivo* and *in vitro* evidences that morin is a potent neuroprotective agent in a model of PD. Further study will be focused on revealing neuroprotective mechanism of morin.

Disclosures: J. Lee: None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

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Topic: C.03. Parkinson's Disease

Support: Botany in Action fellowship (Phipps botanical garden, Pittsburgh)

NIH Grant R21 AG039718

NIH Grant 1R03 DA027111

Title: Traditional medicines: mechanisms of neuroprotection and the Nrf-2 antioxidant pathway in Parkinson's disease models

Authors: *A. JACQUET, S. Y. MA, J.-C. ROCHET;
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Abstract: As our population ages, the economical and social burden of neurodegenerative diseases such as Parkinson's disease (PD) affects an increasing number of families. Despites global efforts, there are still no treatments to stop or slow the neurodegenerative process underlying PD. We therefore initiated an ethnopharmacological study to identify medicinal plants used in Nepalese and Native American traditional medicine to treat PD and PD-related symptoms. Among the 50+ plants documented, we identified six promising candidates including the isoflavone-rich red clover extract. The neuroprotective activities of these extracts were assessed in immortalized and primary neuronal cell cultures. We showed that our plant extracts (including red clover) and individual isoflavones alleviate toxicity elicited by rotenone, paraquat and/or the α-synuclein mutant A53T as determined by monitoring dopaminergic neuron survival and the preservation of neurite lengths. The plant extracts showed the ability to increase the cellular antioxidant response via activation of the Nrf-2/ARE pathway, as determined in primary cortical astrocytes and mixed primary midbrain cultures transduced with an Nrf-2 reporter adenovirus encoding EGFP downstream of the antioxidant response element (ARE). The extracts also interfered with a loss of mitochondrial function triggered by the complex I inhibitor rotenone in SH-SY5Y cells maintained in galactose-containing media to ensure that they were dependent on oxidative phosphorylation for their energy production. Finally, the red clover extract was found to inhibit proteasomal function as monitored using GFP-U, an unstable form of GFP, as a substrate protein to study ubiquitination-dependent proteasomal degradation. Current efforts are aimed at determining whether the inhibitory effect of the red clover extract on the ubiquitin-proteasome system results in an activation of lysosomal autophagy. Collectively, our results suggest that red clover and other polyphenol-rich botanical extracts protect against toxicity elicited by PD-related insults by activating the cellular antioxidant response, ameliorating mitochondrial dysfunction, and modulating protein degradation. These insights about the neuroprotective mechanisms of botanical extracts take us a step closer to the design of herbal remedies for PD. With 80% of the world population using medicinal plants as a primary source of healthcare, the development of plant-based therapies would critically impact the lives of these patients.

Disclosures: **A. Jacquet:** None. **S.Y. Ma:** None. **J. Rochet:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Title: Neuroprotective effect of beta-caryophyllene in Parkinsonism model with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on adult C57BL/6 mice

Authors: *H. A. SUCRES-BERNES[1], J. M. VIVEROS-PAREDES[2], V. CHAPARRO-HUERTA[3], R. E. GOZALEZ-CASTAÑEDA[4], C. R. GARCIA-LEMUS[5], M. E. FLORES-SOTO[5];
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Abstract: The Parkinson's Desease (PD) is a neuromotor disorder characterized by the lost of the dopaminergic cells, specifically in the substance nigra (SNpc) pars compacta in mild brain. Nigral dopaminergic cells are particularly vulnerable to oxidative stress which is a key stimulator of microglia activation, which produce reactive oxygen species (ROS) from microglia, and the subsequently dopaminergic cells death to propagate and propel feed forward cycle of neuronal death and inflammation underlying the progression of cell death. Exist evidence that the inflammation which are produced by microglia activation amplify the cell death.In this years, the cannabinoid CB2 receptor (CB2r) , has a new roll like a pharmacological target by the modulation of neuroinflammation because it is upregulated on activated microglia an has a numerous functional effects on these cells. Beta-Caryophyllene is a strongly CB2 agonist which has emerged in recent years. We investigate if beta-caryophyllene can modulate the neuroinflammation response by activation of CB2r in Parkinsonism. We administrated v.i.p MPTP (30mg/kg/d) for 3 days, and beta-caryophyllene (10mg/kg/d) 5 days before MPTP. Groups were: CTL, MPTP, B+MPTP, Beta. After 72h of treatment we sacrificed by decapitation or perfusion, quickly remove SNpc and striatium. Inmunohistochemistry with tyrosine hydroxilase (TH), Iba1 and GFAP. Quantify levels by ELISA of TNFa, IL-6, IL-1b which are related with inflammation. We obtained that immunoreactivity of TH in MPTP group decrease in SNpc and striatum, but this were revert in the beta-caryophyllene group. The inmunoreactivity of Iba1 and GFAP increase in MPTP group and significantly decrease on beta-caryophyllene group. Our results suggest that mice treated with beta-caryophyllene were significant different compared whit the other groups for all techniques.

Disclosures: **H.A. Sucres-Bernes:** None. **J.M. Viveros-Paredes:** None. **V. Chaparro-Huerta:** None. **R.E. Gozalez-Castañeda:** None. **C.R. Garcia-Lemus:** None. **M.E. Flores-Soto:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: Cure Parkinson's Trust grant (CPT)

Title: Is Liraglutide effective in 6-hydroxydopamine induced rat model of Parkinson's disease?

Authors: ***M. K. SHARMA**, J. JALEWA, C. HOLSCHER;
Lancaster Univ., Lancaster, United Kingdom

Abstract: Type 2 diabetes is a risk factor in the development of Parkinson's disease (PD) and the insulin signalling in the brains of PD patients is impaired. Currently, the treatments available cannot repair or prevent further damage in the brain, thus there is urgent need to develop novel treatments. Liraglutide (Victoza®), a long-lasting GLP-1 analogue is on the market as treatment for diabetes. Previous research has shown neuroprotective and growth factor properties of Liraglutide in the brain. Liraglutide crosses the blood-brain barrier and increases synaptic plasticity and cognitive function. The current study aims to answer whether Liraglutide will exhibit neuroprotective effects in 6-OHDA induced rat model of PD. Sprague Dawley rats were injected with Liraglutide (25nm/kgbw) or saline (0.9%w/v). The effect of Liraglutide treatment will be assessed on motor behaviour using rotarod and grip strength meter. In addition, we will study the dynamic gait signals of the rat on Digigait and analyse the parameters of animal posture and kinematics. Tyrosine hydroxylase immunopositive neurons will be counted in the substantia nigra (SNpc) and striatum (caudate putamen). Here, for the first time we test the effect of intraperitoneal (i.p.) administration of Liraglutide as a treatment for the motor impairment suffered by 6-hydroxydopamine (6-OHDA) injected rat PD models. Results will be disclosed at the meeting.

Disclosures: **M.K. Sharma:** None. **J. Jalewa:** None. **C. Holscher:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: NIH Grant P20GM103395

Title: Alaskan blueberry and Sirtuin 1-mediated neuroprotection in a Caenorhabditis elegans model of Parkinson's disease

Authors: *M. MAULIK[1,2], S. KUHN[3], B. E. TAYLOR[4];
[2]Dept. of Chem. and Biochem., [3]Dept. of Biol. and Wildlife, [4]Dept. of Biol. and Wildlife, Dept. of Chem. and Biochem., [1]Univ. of Alaska Fairbanks, Fairbanks, AK

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons and aggregation of alpha-synuclein (AS) protein leading to motor and cognitive impairment. The current study investigates the role of Alaskan bog blueberry (*Vaccinum uliginosum),* on alpha synuclein aggregation using a transgenic model of *Caenorhabditis elegans* expressing human alpha-synuclein [NL5901 (P(unc-54)::alpha-synuclein::YFP+unc-119)].The current study also examines the role of Sirtuin 1, a histone deacetylase, in reducing the toxicity of alpha-synuclein aggregates and whether this effect is mediated via expression of molecular chaperones, HSP1 and HSP70. The Alaskan bog blueberry was chosen for its high phenolic content, because phenolics have been shown to modulate sirtuin-mediated molecular pathways. Our experiments showed that the crude extract of low bog blueberry (400 and 800 ug/ml) reduced alpha-synuclein aggregation. For high-dose blueberry extract (800 ug/ml), the protection was mediated by sirtuin and HSF-1, which is independent of HSP 70. These findings encourage further studies on these Alaskan botanicals as possible therapeutic agents for Parkinson's disease, specifically of interest are the identification of active ingredients within the extracts and their optimal doses.

Disclosures: M. Maulik: None. **S. Kuhn:** None. **B.E. Taylor:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

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Program#/Poster#: 678.20/E30

Topic: C.03. Parkinson's Disease

Support: The Innovative Research and Development Program (Texas State)

Title: N-acetyl-cysteine in combination with igf-1 enhances neuroprotection against proteasome dysfunction-induced neurotoxicity in sh-sy5y cells

Authors: *B. CHENG[1], A. ROMAN[2], A. KUANG[2], E. CASILLAS[1];
[1]Univ. Texas Hlth. Sci. Ctr., Edinburg, TX; [2]Biol., UT-PAN-American, Edinburg, TX

Abstract: The ubiquitin proteasome system (UPS) dysfunction has been implicated in the development of many neuronal disorders, including Parkinson's disease (PD). Previous studies focused on individual neuroprotective agents and their respective abilities to prevent neurotoxicity following a variety of toxic insults. However, the effects of the antioxidant N-acetyl cysteine (NAC) on proteasome impairment-induced apoptosis have not been well characterized in human neuronal cells. The aim of this study was to determine whether co-treatment of NAC and insulin-like growth factor (IGF)-1 efficiently protected against proteasome inhibitor-induced cytotoxicity in SH-SY5Y cells. Our results demonstrate that the proteasome inhibitor, MG132, initiates poly (ADP) ribose polymerase (PARP) cleavage, caspase-3 activation, and nuclear condensation and fragmentation. In addition, MG132 treatment leads to endoplasmic reticulum (ER) stress and autophagy-mediated cell death. All of these events can be attenuated without obvious reduction of MG132-induced protein ubiquitination by first treating the cells with NAC and IGF-1 separately, or simultaneously prior to exposure to MG132. Moreover, our data demonstrated that the combination of the two, rather than used individually, proved to be significantly more effective for neuronal protection. Therefore, we conclude that the simultaneous use of growth/neurotrophic factors and a free radical scavenger may increase overall protection against UPS dysfunction-mediated cytotoxicity and neurodegeneration.

Disclosures: B. Cheng: None. **A. Roman:** None. **A. Kuang:** None. **E. Casillas:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

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Topic: C.03. Parkinson's Disease

Support: NRF31M099

Title: Beta-Caryophyllene ameliorates oxidative stress and neuroinflammation in rat model of rotenone-induced Parkinson's disease

Authors: *S. K. OJHA[1], C. SHARMA[2], H. JAVED[3], S. AZIMULLAH[4], S. BEGUM[3], E. HAQUE[3];
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Abstract: Parkinson disease (PD) is the second most common neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia niagra (SNc) of brain. A large number of studies demonstrate that oxidative stress and inflammation play a critical role in the etiopathogenesis of PD. The current study was undertaken to determine the neuroprotective potential of beta-caryophyllene (BCP), a phytocannabinoid and natural sesquiterpene of wide occurrence. Recent approved by FDA for its use as food additive and flavoring agent encouraged investigated its medicinal properties. Following the chronic and progressive nature of PD pathogenesis, BCP (50 mg/kg) and rotenone (3 mg/kg) were administered to rats for four weeks, as rotenone-induced PD recapitulates human PD pathogenesis. Rotenone challenge caused a significant decrease in activities of antioxidants as evidenced by reduced superoxide dismutase, catalase and reduced glutathione with a concomitant increase in the lipid peroxidation product, malondialdehyde. Rotenone injections also significantly induced pro-inflammatory cytokines; IL-1β, IL-6 and TNF-α and enhanced the expression of inflammatory mediators; cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in the midbrain. Immunohistochemical studies reveal a significant increase in the expression of ionized calcium binding adaptor molecule-1 (Iba-1) and glial fibrillary acidic protein (GFAP) indicative of microglia activation and astrocyte activation, respectively with loss of dopamine neurons in the SNc following rotenone exposure. However, in our study, treatment with BCP significantly protected the dopaminergic neurons, improved antioxidant enzymes and stabilized the activation of Iba-1 and GFAP resulted from the rotenone challenge. BCP also prevented glutathione depletion and inhibited lipid peroxidation along with significant attenuation of the induction of pro-inflammatory cytokines. Further, BCP treatment also attenuated the increased levels of other inflammatory mediators; COX-2 and iNOS. Based on the results of the present study, it is concluded that BCP protects against rotenone-induced neurodegeneration in PD. The neuroprotective effects of BCP, a naturally available phytocannabinoid could be promising for neuroprotection and the neuroprotective potential could be attributed to its potent antioxidant and anti-inflammatory properties.

Disclosures: **S.K. Ojha:** None. **C. Sharma:** None. **H. Javed:** None. **S. Azimullah:** None. **S. Begum:** None. **E. Haque:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.22/E32

Topic: C.03. Parkinson's Disease

Support: JSPS KAKENHI 25830053

JSPS KAKENHI 24300134

Title: Necdin promotes mitochondrial biogenesis to prevent neurodegeneration in experimental Parkinson's disease

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Abstract: Mitochondria play a pivotal role in neuronal survival and function. Mitochondrial dysfunction causes neurodegenerative diseases such as Parkinson's disease (PD). Necdin is predominantly expressed in postmitotic neurons and promotes neuronal differentiation and survival. We have previously shown that necdin binds to Sirt1, an NAD+-dependent protein deacetylase involved in the regulation of energy homeostasis and cell survival, and facilitates Sirt1-mediated deacetylation of its substrates p53 and FoxO1 in neurons (J Neurosci, 28:8772-8784, 2008; ibid., 32:5562-5572, 2012). In the present study, we examined whether necdin affects neuronal mitochondrial biogenesis using necdin-null mice. Microarray-based gene expression profiling and quantitative real-time PCR analysis revealed that expression of mitochondria-specific and mitochondrial biogenesis-related genes was significantly low in necdin-null cortical neurons. Furthermore, the amounts of mitochondria and ATP were reduced in necdin-null neurons, which expressed low protein levels of PGC-1α, a master regulator of mitochondrial biogenesis and function. Coimmunoprecipitation assay revealed that necdin bound and stabilized PGC-1α by preventing its ubiquitin-dependent degradation. Forced expression of necdin in primary cortical neurons and human neuroblastoma SH-SY5Y cells promoted mitochondrial function via PGC-1α stabilization and suppressed their mitochondrial toxin-induced degeneration. Furthermore, necdin gene transfer into the substantia nigra *in vivo* of adult mice prevented MPTP-induced degeneration of dopaminergic neurons. These results suggest that necdin stabilizes PGC-1α to promote neuronal mitochondrial biogenesis and prevents mitochondrial dysfunction-associated neurodegeneration.

Disclosures: K. Hasegawa: None. **T. Yasuda:** None. **C. Shiraishi:** None. **K. Fujiwara:** None. **H. Mochizuki:** None. **K. Yoshikawa:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.23/E33

Topic: C.03. Parkinson's Disease

Support: HIgher Education Commission, Goverment of Pakistan

Title: Nigella sativa oil reduces extrapyramidal side effects (EPS)-like behavior in haloperidol-treated rats

Authors: *T. MALIK[1,2], S. HASAN[3], S. PERVEZ[4], T. FATIMA[5], D. J. HALEEM[6], H. DONG[2];
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Abstract: The symptoms of Parkinsonism and oral dyskinesia have been showing to be induced by neuroleptics that significantly affect its clinical use. In this study, we investigate whether Nigella sativa-oil (NS) (black cumin seeds) - a traditional medicine used for the seizure treatment in eastern country- may reduce the haloperidol (HAL) - induced extrapyramidal symptoms (EPS) - like behavior in rats. After combine treatment with HAL (1mg/kg) on NS (0.2ml/rat), rats displayed a significant decreased EPS-like behavior including movement disorders and oral dyskinesia as compared to controls. Immunohistochemical analysis indicates that NS reduced astrogliosis in Caudate and Accumbens Nuclei. These results suggest that NS may consider as an adjunct to antipsychotics to reduce the EPS-like side effect. **Figure 1: Striatal Histopathology (GFAP) at the Dorso-Lateral Region of Caudate Figure: 2 Active Microglia in the Bilateral Striata.**

Disclosures: **T. Malik:** 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.; Higher Education Commission, Goverment of Pakistan. **S. Hasan:** None. **S. Pervez:** None. **T. Fatima:** None. **D.J. Haleem:** None. **H. Dong:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

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Program#/Poster#: 678.24/E34

Topic: C.03. Parkinson’s Disease

Support: Korea Health Technology R&D Project through the KHIDI Grant HI14C1913

NRF Grant 2005-0093836

Asan Institute for Life Science Grant 2015-624

Dong-A ST

Title: DA-9805, mixture of herbal extracts, promotes the degradation of α-synuclein in SH-SY5Y cells via autophagy activation

Authors: *H.-R. BYUN[1], H. KIM[1], J. KIM[1], J. KOH[2];
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Abstract: Accumulation of α-synuclein in Lewy bodies and neurites is a pathological hallmark of Parkinson disease (PD). Mutations in α-synuclein cause familial PD, suggesting that altered α-synuclein function can trigger the neurodegenerative process. The formation of cytoplasmic α-synuclein pathologies in PD has been attributed to the dysfunction of protein degradation pathways such as proteasome and autophagy/lysosome systems. DA-9805 is a mixture of herbal extracts prepared by Dong-A ST with PD as a disease target. Although precise pharmacological mechanisms are yet to be characterized, its targets in PD models seem to include inflammation, oxidative stress and autophagy. One of the factors that regulate autophagy flux may be cAMP that has been reported to boost autophagy flux. Of interest, a significant decrease in cAMP levels was observed in patients with PD (Nishino et al. Rev. Neurosci. 1993). Since preliminary data suggested that DA-9805 interacted with PDE1, in the present study, we examined the possibility that DA-9805 reduces α-synuclein accumulation in SH-SY5Y cells through the activation of autophagy. As we predicted, DA-9805 inhibited PDEs and increased cAMP levels in SH-SY5Y cells. Indicating that DA-9805 thus increased autophagy flux, it decreased levels of LC3 and p62 in SH-SY5Y cells. Furthermore, it promoted α-synuclein protein degradation in an autophagy-dependent manner. Present results indicate that DA-9805, likely by increasing the cAMP levels, is an activator of autophagy in SH-SY5Y cells, and by this mechanism, may reduce α-synuclein accumulation. Taken together, our results suggest that DA-9805 may prove useful as a therapeutic agent against neurodegenerative proteinopathies such as PD and AD.

Disclosures: **H. Byun:** None. **H. Kim:** None. **J. Kim:** None. **J. Koh:** None.

Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.25/E35

Topic: C.03. Parkinson's Disease

Support: France Parkinson

Fondation philanthropique Edmond J. safra

Crédit Agricole Sud Rhône-Alpes

Institut Carnot

Title: Because it does not influence MAO-B and DAT activities, the Near Infrared light therapy is a promising protective strategy against Parkinson's disease

Authors: F. REINHART[1,2], F. DARLOT[1,2], C. CHABROL[1,2], C. GAUDE[1,2], D. RATEL[1,2], N. TORRES-MARTINEZ[1,2], A.-L. BENABID[1,2], *C. MORO[1,2];
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Abstract: Parkinson's disease (PD) is a major movement disorder and neurodegenerative disease. The current therapies are effective against symptoms but cannot protect the patient against the cells degeneration. It exists several preclinical models using toxins or genetic that can mimic the parkinsonian physiopathology and help us to find new therapies agains PD. The most used or them, the 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) is well known to mimic the human PD because of its capacity to specifically intoxicate the dopaminergic structures, like in substancia nigra pars compacta. Firstly, the MPTP is oxidised in MPP+ (which is the neurotoxic form of MPTP) by the monoamine oxidase B (MAO-B). Secondly, the MPP+ crosses the plasmic membrane of the dopaminergic cells through the dopamine transporter (DAT). Obviously, any neuroprotective strategy tested in these different models must be proven to not modulate neither the MAO-B activity, nor the DAT activity. We assess here that a new neuroprotective strategy using near infrared (NIr) illumination is not influencing the MAO-B and DAT activities. Indeed, a growing number of recent studies in MPTP models of Parkinson's disease have reported that a NIr light therapy (λ = 600-1000 nm) can be neuroprotective in PD. The MAO-B and DAT activities have been measured *in vitro* respectively by the kits MAO-Glo assay® (PromegaTM) and Neurotransmitter Transporter Uptake Assay Kit® (Molecular

DeviceTM). Briefly, the principles are to present the MAO-B or the DAT to a substrate coupled with a blinded luminophore (MAO-B) or fluorophore (DAT). After the reaction, we can measure the quantity of luminophore or fluorophore freed by a measurement of the light emitted by the medium of interest, and thus to deduce the MAO-B or DAT activities. We had several conditions here: (1) MAO-B or DAT + substrate in darkness and (2) MAO-B or DAT + substrate under different NIr illuminations. We show here that a NIr exposure, used as a therapeutical strategy against the PD does not modulate neither the MAO-B, nor the DAT activity. Thus, the results demonstrating a protective effect of NIr therapy in MPTP models are actually due to a real neuroprotective effect and not to an experimental bias. Our results confirm the interest of a NIr therapy against the disease and allow us to seriously consider this strategy in human.

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Poster

678. Therapeutics of Parkinson's Disease: Neuroprotection

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 678.26/E36

Topic: C.03. Parkinson’s Disease

Support: MRC

Title: Neuroprotective effect of Korea Red Ginseng extract on 1-methyl-4-phenylpyridinium ion (MPP^+)-induced apoptosis in PC12 cells

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Abstract: Red ginseng is valuable herb in Asian countries as a crude substance to enhance vitality, health and longevity. The water extract of red ginseng has been used to promote immunity and inhibiting inflammation. To further explore its actions, the present study evaluated protective effects of Korean red ginseng (KRG) extract against 1-methyl-4-phenylpyridine (MPP^+)-induced apoptosis in dopaminergic neurons. KRG extract was pretreated on PC12 cells for 24hours prior to MPP^+ expose to cells and live cell viability assay, flow cytometry, quantitative real-time PCR, and TUNEL staining were performed on PC12 neuronal cells. KRG treatment significantly enhance cell viability, reduced Annexin V-FITC and propidium iodide double staining cells and TUNEL-positive cells indicated that KRG treatment reduced MPP^+-

induced apoptosis in PC12 cells. KRG treatment also reduced caspase-3, -8, and -9 activities. These results suggest that KRG treatment exert a protective effect against MPP+-induced apoptosis in dopaminergic neurons.

Disclosures: S. Ryu: None. **S.T. Kim:** None.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.01/E37

Topic: C.06. Developmental Disorders

Support: NIH Pre-doctoral Training Grant 5T32 GM008541 (MW)

PhRMA Foundation Pre-doctoral Fellowship in Pharmacology & Toxicology (MW)

Nancy Lurie Marks Family Fund (TI)

Landreth Family Foundation (TI)

Title: Longitudinal characterization of microglial gene expression profile and behavioral, morphological and neurophysiological abnormalities in a mouse model of autism spectrum disorder

Authors: *M. E. WOODBURY[1], S. IKEZU[2], J. I. LUEBKE[3], P.-H. CHAO[2], T. IKEZU[2]; [1]Grad. Program in Neuroscience; Pharmacol. and Exptl. Therapeut., [2]Pharmacol. and Exptl. Therapeutics; Neurol., [3]Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: Mounting evidence implicates a role of peripheral and central innate immunity in neuropsychiatric disorders, including autism spectrum disorder (ASD). Microglia, the innate immune cells of the CNS, show changes in density, morphology, and/or activation phenotype in post-mortem ASD brains and recently by [11C] PK11195 PET imaging of young patients with ASD. Maternal immune activation (MIA) is significantly associated with higher incidence of autism in children in multiple large-cohort studies. We hypothesize that MIA dysregulates microglial homeostasis of the offspring, which in turn leads to spine dysgenesis, abnormal neuronal excitability, and ASD-like behavior. Using a MIA animal model induced by intraperitoneal injection of the innate immunity ligand polyinosinic:polycytidylic acid [poly(I:C)],on embryonic day 9.5 (E9.5), we profiled microglial gene expression in the offspring at several time points and associated this with behavioral and neuroanatomical findings as a

longitudinal study. We found that microglia highly expressed molecules associated with neural differentiation, neurite outgrowth, and synaptogenesis, which were enhanced at prenatal and postnatal time points in offspring following poly(I:C) injection to pregnant dams. *In vitro,* knockdown of key molecules in microglia was associated with reduced neural differentiation, neurite outgrowth and expression of synaptic markers in co-cultured neural precursor cells, assessed using transwells and our custom microfluidic cell culture system. Following dysregulation of microglial gene expression at embryonic and early postnatal stages, MIA induced ASD-like behavior in the offspring, including reduced maternal approach and decreased sociability, observed from postnatal week 3 to 8. Whole-cell patch clamp recordings of prefrontal layer V cortical pyramidal neurons at 8 weeks of age revealed enhanced excitatory synaptic activity as evidenced by increased frequency and reduced amplitude of miniature and spontaneous excitatory postsynaptic currents. This was associated with increased spine density and abnormal spine subtype distribution in apical dendrites of biocytin-filled layer V cortical pyramidal neurons, assessed by laser-scanning confocal microscopy. Our findings highlight the role of microglia in ASD pathogenesis, and suggest that microglial gene expression is a promising target for pharmacological intervention in ASD.

Disclosures: M.E. Woodbury: None. **S. Ikezu:** None. **J.I. Luebke:** None. **P. Chao:** None. **T. Ikezu:** None.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.02/E38

Topic: C.06. Developmental Disorders

Title: Cytoplasmic function of AUTS2 in neural network formation

Authors: *M. HOSHINO[1], T. NAGAI[2], W. SHEN[2], A. SAKAMOTO[1], S. TAYA[1], R. HASHIMOTO[1], T. HAYASHI[1], M. ABE[3], M. YAMAZAKI[3], K. NAKAO[4], T. NISHIOKA[2], K. SAKIMURA[3], K. YAMADA[2], K. KAIBUCHI[2], K. HORI[1];
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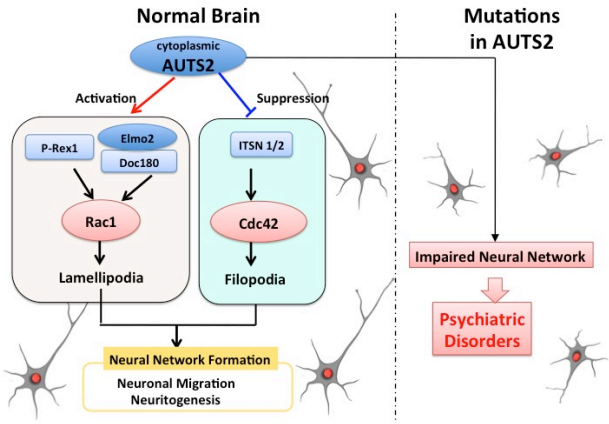
Abstract: Mutations in the *Autism susceptibility candidate 2 gene* (*AUTS2*), whose protein is believed to act in neuronal cell nuclei, have been shown to be associated with multiple psychiatric illnesses including autism spectrum disorders (ASD), intellectual disability (ID), schizophrenia, epilepsy and ADHD. Here we reveal a novel function of cytoplasmic AUTS2 in

the regulation of cytoskeleton and neural development. Immunohistochemistry and fractionation studies show that AUTS2 localizes not only in nuclei, but also in the cytoplasm, including growth cones in the developing brain. AUTS2 activates Rac1 to induce lamellipodia via interacting with P-Rex1 and Elmo2/Dock180, both of which are guanine nucleotide exchange factors (GEF) for Rac1. On the other hand, AUTS2 downregulates Cdc42 to suppress filopodia via interacting with Intersectin 1 and Intersectin 2, specific GEFs for Cdc42. Our loss-of-function and rescue experiments show that a cytoplasmic AUTS2-Rac1 pathway is involved in cortical neuronal migration and neuritogenesis in the developing brain. Moreover, *Auts2*-deficient mice display behavioral abnormalities including anxiety-related emotion and memory formation. These findings suggest that cytoplasmic AUTS2 acts as a novel regulator for Rho family GTPases contributing to brain development and give good insights into the pathology of human psychiatric disorders with *AUTS2* mutations.



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Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.03/E39

Topic: C.06. Developmental Disorders

Title: Development of dendritic structure in the btbr mouse model of autism

Authors: F. ALSHAMMARI[1], N. CHENG[1], M. KHANBABAEI[1], E. HUGHES[1], R. TOBIAS[1], *J. M. RHO[2];
[1]Cumming Sch. of Medicine, Univ. of Calgary, Calgary, AB, Canada; [2]Alberta Children's Hospital, Univ. of Calgary, Calgary, AB, Canada

Abstract: Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder that is increasing in prevalence and is defined by impaired sociability, deficits in communication skills, and restricted and/or stereotyped behaviors. Recent studies involving young ASD patients (between 1-3 years old) have shown larger brain volumes compared to age-matched controls, suggesting that early neurodevelopment may be altered and may contribute to disease pathogenesis. Here, we investigated the development of dendritic arbors and neuronal densities of hippocampal pyramidal neurons in the BTBR mouse model of autism, which displays all three core behavioral symptoms of this disorder. Our preliminary studies using Golgi staining revealed that at the neonatal stage (postnatal day 8), the total lengths of both apical and basal dendrites of pyramidal neurons in the hippocampal CA1 region of the BTBR animals were greater than that in the B6 control animals, a strain commonly used as a control for the BTBR animals in ASD studies (B6 total apical: 298±20 μm, total basal: 330±43 μm; BTBR total apical: 381±18 μm, total basal: 401±28 μm). Dendritic branching was further analyzed by using Sholl analysis that measures the number of dendrites crossing concentric circles as a function of distance from the soma. Preliminary results revealed that there were more dendritic intersections at 40 to 60 mm distances from the soma in the BTBR compared to B6 animals (B6: 4.3±0.5 intersections at 40 mm and 1.8±0.5 intersections at 60 mm; BTBR: 5.4±0.4 intersections at 40 mm and 2.9±0.3 intersections at 60 mm). In contrast, Nissl staining demonstrated a similar density of CA1 pyramidal neurons between the BTBR and B6 pups (B6: 0.0072±0.0003 cells/μm^2; BTBR: 0.0068±0.0003 cells/μm^2), and a similar thickness of the CA1 pyramidal neuron layer between the two strains (B6: 106±2 μm; BTBR: 100±2 μm). At the adult stage, the thickness of the pyramidal neuron layer was reduced compared with neonates (B6: 52±1 μm; BTBR: 51±0.5 μm), whereas the densities of pyramidal neurons remained similar between the BTBR and C57 animals (B6: 0.0075±0.0004 cells/μm^2; BTBR: 0.0085±0.0003 cells/μm^2). Further experiments to quantify dendritic structure in adult animals are required to determine whether the BTBR model has larger and more complex dendritic arbors, or whether the development of dendritic arbor is accelerated in these animals. Considering the significance of dendritic structure in information processing, our results suggest that altered dendritic structure may contribute to abnormal behaviors seen in this animal model of ASD.

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Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Topic: C.06. Developmental Disorders

Support: MD-II-2013-269

R21 MH099798-01

R21 NS083052-02

R21 DA038458-01

Title: RNA-binding protein Celf6 regulates actively translated mRNAs enriched in neuromodulatory neurotransmitter cell populations

Authors: *M. A. RIEGER[1], J. D. DOUGHERTY[2];
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Abstract: A growing body of literature has highlighted the importance of RNA-binding proteins (RBPs) in the development and maintenance of the central nervous system. The functional roles of these proteins are implicated in the etiologies of psychiatric disorder and neurodegenerative disease. The mechanism by which RBPs regulate their targets is diverse: including splicing, stability, localization and rate of translation. These in turn have consequences for neuronal cellular phenotypes such a differentiation and synaptic plasticity. Our laboratory uses the Translating Ribosome Affinity Purification (TRAP) methodology which allows us to profile mRNAs in a cell-type specific manner. Using TRAP to identify mRNA species enriched in serotonergic neurons, we identified the RNA-binding protein Celf6 and later found it to be associated with autism spectrum disorder risk. A homozygous null mutation in this gene in mice presented with reductions to both post-natal ultrasonic vocalization behavior and levels of neuromodulatory neurotransmitters such as serotonin. To understand the molecular role of Celf6 in serotonergic neurons, we profiled these cells using TRAP in the context of a Celf6 null animal. The abundance of a number of mRNAs on ribosomes were found to differ between Celf6 wild-type and null animals using this approach. We examined these species for commonalities at the level of sequence and structure. These mRNAs contained cytosine and uracil-rich stem-loop motifs identified in silico within the 3' untranslated region (3' UTR). To better understand their regulation, we are next turning to an *in vitro* culture system. Using cells expressing epitope-tagged Celf6 we will be able to determine the specificity of interaction between Celf6 and putative targets by cloning 3' UTRs into a fluorescent reporter and assessing the Celf6-dependence of reporter levels as well as evidence of direct binding using cross-linking

immunoprecipitation. We will test whether structural integrity of motifs in the 3' UTR affects mRNA stability and efficiency of translation in a Celf6-dependent manner. Using the information obtained *in vitro*, we can then make predictions as to which mRNAs would be differentially regulated in an independent population of cells and use TRAP to validate these predictions. Taken together we outline a pipeline of techniques that powerfully combines *in vivo* and *in vitro* methods. Using these methods we hope to gain insight into the role of key regulatory molecules in the brain that affect behavior and cellular function.

Disclosures: M.A. Rieger: None. **J.D. Dougherty:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TRAP licensing.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.05/E41

Topic: C.06. Developmental Disorders

Support: HHMI foundation

Simons Foundation

Title: Regulation of synaptic function in hippocampal CA1 pyramidal cells by neuroligins

Authors: *M. JIANG[1], J. S. POLEPALLI[2], R. C. MALENKA[2], T. C. SÜDHOF[1,3];
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Abstract: Neuroligins (NL) are postsynaptic adhesion molecules, which have been implicated in autism spectrum disorders (ASD). Despite a large body of work, their detailed synaptic functions remain unknown and controversial. To begin to address the requisite synaptic functions of NLs precisely, we generated mutant mice carrying conditional knockout (cKO) alleles of the three major NLs (NL1, 2, and 3). Injection of viruses expressing Cre *in vivo* at postnatal day 0 (P0) and day 21 (P21) followed by acute slice electrophysiology ~2 weeks later allowed us to study the consequences of genetically deleting NL1-3 *in vivo* at different developmental stages on inhibitory and excitatory synaptic function in hippocampal CA1 pyramidal neurons. Deletion of NL1-3 at P0 caused a clear decrease in inhibitory synaptic transmission; an effect that was

phenocopied by deletion of NL2 alone. In contrast, P0 deletion of NL1-3 had no detectable effect on AMPA receptor-mediated miniature EPSCs and no effect on dendritic spine density. However, triple NL1-3 cKO as well as NL1 cKO alone (but not NL3 cKO) at both P0 and P21 significantly decreased NMDA receptor-mediated synaptic responses. In addition, NL1 cKO but not NL3 cKO abolished long term potentiation (LTP) at both P0 and P21. Several lines of evidence suggest that the block of LTP by NL1 deletion is not due to the impairment in NMDA receptor-mediated synaptic function. These results confirm a critical role for NL2 in inhibitory synapse formation, a critical role for NL1 in maintaining normal NMDA receptor-mediated synaptic transmission, and an unexpected critical role for NL1 in LTP. These single and triple cKO mice will be valuable tools for furthering our understanding of the roles of these ubiquitous postsynaptic cell adhesion proteins in synaptic and circuit function.

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Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.06/E42

Topic: C.06. Developmental Disorders

Support: HHMI foundation

Simons Foundation

Title: The role of Neuroligins on synaptic transmission at Calyx of Held during development

Authors: *B. ZHANG, L. CHEN, T. SUDHOF;
Dept. of Mol. & Cell. Physiol., Stanford medical school, Stanford, CA

Abstract: Autism spectrum disorders (ASDs), also designated as Autism, is a heterogeneous cognitive syndrome characterized by impairment in reciprocal social interaction, impairment with verbal and nonverbal communication, and repetitive behaviors with narrow interests. Genome-wide associate studies have identified transsynaptic cell adhesion molecules neuroligins (NLs) are associated with autism. Neuroligins (NLs) are synaptic cell adhesion molecules, includes family members NL 1, -2, -3, and -4 in rodent. NL1 and NL3 function at excitatory synapse, where NL 2 and NL3 function at inhibitory synapse, while NL4 function in glycinergic synapse. Due to its perinatal lethal phenotype of constitute triple knockout NL1/2/3, it is impossible to study the role of NLs at a synapse during the development with this constitute

NL1/2/3 triple KO mice. Besides, knockdown strategy used for determining the role of NLs suffer from off-target effect. Therefore, the role of NLs in synapse formation/maturation in a mammalian CNS synapse is largely unknown. The calyx of Held in the auditory system is a giant and fast-transmitting synapse with hundreds of active zones covering half of the postsynaptic principal neuron in the medial nucleus of the trapezoid body. The development of calyx of Held synapses is characterized sequentially by, 1): an initially small glutamatergic nerve terminals contacted by several fibers, 2), the emerging of protocalyces after elimination of redundant synapses being, and 3), calyces formed with faster transmitter release and postsynaptic receptor kinetics. However, the molecular mechanisms that drive synapses elimination and synapse maturation of the calyx of Held synapses, remain largely unknown. Here we used the large calyx of Held synapse as a model synapse to study the role of NLs in synapse formation/maturation in the mammalian CNS. Using mouse genetics (conditional NLs knockout mice), stereotactic virus injection, electrophysiology, and immunostaining, we demonstrate a specific and essential role of NLs for the development of these large auditory relay synapses.

Disclosures: B. Zhang: None. **L. Chen:** None. **T. Sudhof:** None.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.07/E43

Topic: C.06. Developmental Disorders

Support: New Jersey Governor's Council for Medical Research and Treatment of Autism (PI: Millonig)

New Jersey Governor's Council for Medical Research and Treatment of Autism CAUT14APL031 (PI: DiCicco-Bloom)

Title: Leveraging iPSCs to define molecular mechanisms of autism

Authors: *J. H. MILLONIG[1], P. MATTESON[2], P. YEUNG[3], M. WILLIAMS[4], S. PREM[4], Z. PANG[3], L. BRZUSTOWICZ[5], C.-W. LU[3], E. DICICCO-BLOOM[4];
[1]Neurosci. and Cell Biol., Rutgers-Rwjms, Piscataway, NJ; [2]Ctr. for Advanced Biotech. and Med., Rutgers RWJMS, Piscataway, NJ; [3]Child Hlth. Inst. of NJ, Rutgers RWJMS, New Brunswick, NJ; [4]Neurosci. and Cell Biol., Rutgers RWJMS, Piscataway, NJ; [5]Genet., Rutgers U, Piscataway, NJ

Abstract: The advent of induced pluripotent stem cell (iPSC) technology provides new avenues to generate neural precursors and neurons from patients with CNS disorders. The study of autism etiology has been hampered by both the inability to study human neurons and the heterogeneity of the disorder. While mechanistic analyses in model systems are useful, it is likely that definition of the fundamental pathophysiology will demand study of human neuronal development and function. While iPSC technology was thought of limited use in complex and heterogeneous disorders, we (NJ Autism Center of Excellence; PI Millonig) have now successfully applied these techniques to autism. To reduce heterogeneity between samples, we employed three strategies: 1) families were recruited for language phenotypes including idiopathic autism and another language disorder called Specific Language Impairment; 2) autism cases are compared to unaffected brothers as controls; 3) male sibpairs were deliberately chosen where the affected individual has a narrow autism diagnosis with either no or severely limited language. We have now generated iPSCs from 8 brother-brother sibpairs using Sendai virus expressing the Yamanaka factors. ICC demonstrates that all clones express iPSC markers (TRA160, NANOG, OCT4). iPSC pluripotency was demonstrated by embryoid body formation followed by QRTPCR for lineage-specific markers. To identify developmental phenotypes, neural stem cells (NSCs) and inducible neurons (iNs) have been generated. We have found reproducible differences in NSC development in one family. ICC and QRTPCR demonstrates that the autism NSCs abnormally express genes (e.g. SOX2, ZIC1, ID2) that function in NSC development (P<.001). These NSCs also display additional neurite outgrowth and migration defects (see Prem; Williams)(P<.001). Strikingly some of the NSC phenotypes are rescued by the addition of biologically relevant small molecules (P<.001). For another family ICC for MAP2 and other differentiation markers demonstrate abnormal iN differentiation including dendritic spine deficits. We plan to further analyze molecular and cellular mechanisms underlying these patient-specific phenotypes to identify pathways and targets as well as complete analysis of the 8 sibling pairs in hand.

Disclosures: J.H. Millonig: None. **P. Matteson:** None. **P. Yeung:** None. **M. Williams:** None. **S. Prem:** None. **Z. Pang:** None. **L. Brzustowicz:** None. **C. Lu:** None. **E. DiCicco-Bloom:** None.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.08/E44

Topic: C.06. Developmental Disorders

Title: effect of co-ultramicronized PEALut treatment in a murine model of autism spectrum disorder

Authors: *R. CRUPI[1], D. IMPELLIZZERI[1], G. BRUSCHETTA[1], R. SIRACUSA[1], M. CORDARO[1], E. ESPOSITO[1], S. CUZZOCREA[1,2];
[1]Biol. and Envrn. Sci., Univ. of Messina, Messina, Italy; [2]2Manchester Biomed. Res. Ctr., Univ. of Manchester, Manchester, United Kingdom

Abstract: Autism spectrum disorders (ASDs) are pervasive neurodevelopmental disorders characterized by neurological deficits, especially as related to cognitive function. Although its pathogenesis remains unknown, the major hypothesis at present posits that autism is a multifactorial disorder with a genetic predisposition. Brain inflammation can be a key element in the pathogenesis of neuropsychiatric disorders, including a significant proportion of subjects with ASD. N-palmitoylethanolamide (PEA) is considered to be the parent molecule of ALIAmides, known for its anti-inflammatory, analgesic and neuroprotective properties. PEA inhibits peripheral and central neuroinflammation as well as associated symptomatology. Flavonoids, such as luteolin, possess neuroprotective actions in central nervous pathophysiological conditions. Moreover, the association of PEA with Luteolin (co-ultra PEALut) is more effective in eliciting neuroprotective and anti-inflammatory actions in different models of CNS pathologies, than the molecule taken alone. Our goal was to evaluate the effects evoked by orally administration of co-ultra PEALut in the management of inflammatory and neuroregenerative events associated with ASD using a well established experimental model. A multidisciplinary approach was employed to study: behavioral taks; neuroinflammatory pathways; neurogenesis and neuroplasticity alterations. On P14, C57BL/6 mice were injected with 400 mg/kg sodium valproate. Two different set of experiments were conducted. In the first, on P15 mice were administered with co-ultraPEALut (1mg/kg, daily) and on P30 the behavioral and neuroinflammatory studies were assessed. In the second, at P120, mice started treatment with co-ultraPEALut (2 weeks) and then were sacrified for neurogenesis and neuroplasticity investigations. Co-ultraPEALut ameliorated aggressive behavior and improved spatial learning memory in the valproic acid-induced autistic mice. Moreover, our results revealed the ability of co-ultraPEALut to reduce the level expression of NF-κB, GFAP and nitrotyrosine; also modulate apoptosis in hippocampus and cerebellum. Moreover co-ultraPEALut increases neurogenesis and neuroplasticity in the hippocampus of the autistic mice.

Disclosures: **R. Crupi:** None. **D. Impellizzeri:** None. **G. Bruschetta:** None. **R. Siracusa:** None. **M. Cordaro:** None. **E. Esposito:** None. **S. Cuzzocrea:** None.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Topic: C.06. Developmental Disorders

Support: Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to K.I.)

"Integrated Research on Neuropsychiatric Disorders" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Title: NSF interacts with SERT and modulates its trafficking: implications for path ophysiology in autism

Authors: *K. IWATA[1], H. MATSUZAKI[1], T. TACHIBANA[2], K. NAKAMURA[3], T. KATAYAMA[4], N. MORI[5];
[1]Univ. of Fukui, Yoshida-Gun, Japan; [2]Osaka City Univ., Osaka, Japan; [3]Hirosaki Univ., Hirosaki, Japan; [4]Osaka Univ., Osaka, Japan; [5]Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan

Abstract: Change in serotonin transporter (SERT) function has been implicated in autism. SERT function is influenced by the number of transporter molecules present at the cell surface, which is regulated by various cellular mechanisms including interactions with other proteins. Thus, we searched for novel SERT-binding proteins and investigated whether the expression of one such protein w as affected in subjects with autism. We identified N-ethylmaleimide-sensitive factor (NSF) as a candidate SERT-binding protein by a pull-down system. NSF co-localized with SERT at the plasma membrane, and NSF knockdown resulted in decreased SERT expression at the cell membranes and its uptake function in HEK293-hSERT cells. NSF endogenously co-localized with SERT and interacted with SERT in mouse brain. We measured SERT (SLC6A4) and NSF mRNA expression levels in post-mortem brains and lymphocytes from autistic and control individuals. While SLC6A4 expression was not significantly changed, NSF expression tended to be reduced in post-mortem brains, however this potential trend is not statistically significant, and was significantly reduced and correlated with the severity of the clinical symptom in lymphocytes of autistic subjects. These data clearly show that NSF interacts with SERT under physiological conditions and is required for SERT membrane trafficking and uptake function. A possible role for NSF in the pathophysiology of autism, through modulation of SERT trafficking, is suggested.

Disclosures: K. Iwata: None. **H. Matsuzaki:** None. **T. Tachibana:** None. **K. Nakamura:** None. **T. Katayama:** None. **N. Mori:** None.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.10/E46

Topic: C.06. Developmental Disorders

Support: Alberta Children's Hospital Foundation

Title: Disruption of circuit formation and refinement in a mouse model of autism

Authors: ***N. CHENG**, M. KHANBABAEI, E. HUGHES, R. TOBIAS, K. MURARI, J. M. RHO;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Autism is a complex neurodevelopmental disorder, characterized by deficits in socio-emotional functions and language development, as well as repetitive and/or restrictive behaviours. Because higher-order cognitive abilities are disproportionately affected in autism, it has been hypothesized that disruption in the wiring of the brain, especially in long-range connections, contributes to the pathophysiology of the disease. Here, we investigated circuit formation and refinement during retino-geniculate segregation in the BTBR mouse model of autism, which displays all of the three defining behavioural features of the disorder. The adult lateral geniculate nucleus (LGN) is characterized by its laminated organization into distinct eye-specific layers, which are non-overlapping regions receiving afferents from either the left or the right eye. During normal development, the retino-geniculate afferents from the two eyes are initially overlapping before gradually segregating into eye-specific regions observed at the mature stage. This eye-specific 'map' in the LGN thus has served as a model system to study circuit formation and refinement in the brain. Utilizing this system, we labelled retinal afferents from both eyes of the BTBR mice with an anterograde tracer conjugated with different fluorophores, and compared eye-specific segregation in the LGN between the BTBR model and the B6 animals, a strain commonly used as a control for the BTBR mice in autism studies. We found that in postnatal day 8 animals, the total area of dorsal LGN occupied by retinal afferents from both eyes was significantly smaller in the BTBR mice compared to B6 controls (B6: 0.26±0.04 mm^2, n=5; BTBR: 0.17±0.01 mm^2, n=7; p=0.04). In addition, the degree of overlap between the ipsi- and contralateral afferents was significantly greater in the BTBR than B6 mice. In the B6 animals, the percentage of overlapping area to total dorsal LGN area was between 12.5% to 18.9% using four different thresholds (n=5), while in the BTBR animals, the percentage of overlapping was between 22.2% to 32.3% using the same four thresholds (n=7; p=0.017 to 0.033 corresponding to the four thresholds used). Moreover, these abnormalities in

retino-geniculate input continued into adulthood in the BTBR animals. Taken together, these results indicate that eye-specific projection and segregation in the LGN is impaired in the BTBR animals, suggesting that aberrant circuit formation and refinement may be a feature of this animal model of autism.

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Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.11/E47

Topic: C.06. Developmental Disorders

Title: Characterization of heritable small nucleotide variations in GCN2: implications for autism spectrum disorder

Authors: *A. G. VOROBYEVA[1], A. BHATTACHARYA[1], I. IOSSIFOV[2], T. DEVER[3], E. KLANN[1];
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Abstract: Autism spectrum disorder (ASD) is a heritable neurodevelopmental disorder characterized by the early onset of social deficits, communication impairments, repetitive behaviors, and cognitive inflexibility. Multiple lines of evidence suggest that dysregulated translational control is a common molecular anomaly in ASD pathophysiology. Exome sequencing of families with a child affected by autism revealed numerous heritable autism-associated single nucleotide variations (SNVs) within genes encoding for proteins responsible for controlling protein synthesis. One affected gene is the evolutionarily conserved *EIF2AK4*, which encodes the general control nonderepressible 2 (GCN2) kinase. Upon amino acid starvation, GCN2 preferentially binds uncharged tRNAs resulting in kinase activation and downstream phosphorylation of the α subunit of initiation factor eIF2. The phosphorylation of eIF2 α terminates global translation and enhances the translation of mRNAs containing 5'UTRs with unread open reading frames, including the transcription factor ATF4. We hypothesized that heritable ASD-associated SNVs in *EIF2AK4* alter the kinase function of GCN2. We discovered that multiple heritable SNVs in *EIF2AK4* results in altered GCN2 activity as measured by alterations in eIF2 α phosphorylation, global protein synthesis, and expression of ATF4. Our findings suggest that impaired GCN2 function occurs in multiple individuals with ASD

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Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Topic: C.06. Developmental Disorders

Support: New Jersey Governor's Council for Medical Research and Treatment of Autism (PI: Millonig)

New Jersey Governor's Council for Medical Research and Treatment of Autism CAUT14APL031 (PI: DiCicco-Bloom)

Title: Using exogenous factor treatments to define differences in autism patient-derived neural stem cells

Authors: *M. WILLIAMS[1,2], S. PREM[2], X. ZHOU[2], P. MATTESON[2], P. YEUNG[2], C. LU[2], Z. PANG[2], L. BRZUSTOWICZ[1], J. MILLONIG[2], E. DICICCO-BLOOM[2];
[1]Neurosci., Rutgers Univ., Piscataway, NJ; [2]Neurosci., Rutgers, Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by abnormalities in social interactions and stereotyped/restrictive behavior. The study of ASD has been hindered by disease heterogeneity and difficulties in creating representative mouse models. To examine deficits in sporadic autism, we (NJ Autism Center of Excellence; PI Millonig) generated iPSC lines from 8 severely affected males with ASD and their unaffected brothers (Sib) and derived neural stem cells (NSCs) for study. By applying exogenous factors (EFs) to NSCs we can challenge ASD relevant biological processes such as proliferation and early neuronal maturation. We have examined an array of developmentally relevant EFs including FGF, pituitary adenylate cyclase-activating peptide, BDNF, NT3, insulin, H2O2 and MeHg. Here we focus on FGF due to its role in regulating neurogenesis. To define effects, cells were grown at high density (50K cells/cm2) without and with EFs. At 48h, cells were labeled with tritiated thymidine to assess DNA synthesis and EdU for S phase entry. In parallel, single cell analyses were conducted by acutely dissociating high-density cultures, plating at low density (10K/cm2) and fixed at 2 or 4h for immunostaining. Further, sister cultures were dissociated every 48h for 6 days to quantify live cell numbers via hemocytometer. Comparisons of culture

conditions found that select media and substrate were required to enhance reproducibility and magnitude of response to EFs. As might be expected, FGF induced increases in DNA synthesis and S-phase entry by 50-100% by 48h. Further, FGF mitogenic stimulation at 48h reliably predicted progressive 50% - 100% increases in cell numbers at 4 and 6 days. Conversely, FGF mitogenic stimulation was accompanied by ~60% reductions in proportions of cells expressing β-III tubulin, suggesting FGF maintained the NSC state. Preliminary results in one ASD-Sib pair identified an abnormal ASD-NSC proliferative response, associated with 70% reduction ($p<0.001$) in cellular levels of SOX2 expression. Reduced SOX2 expression may be associated with changes in proliferation as well as restricted lineage differentiation. In aggregate, our results indicate that we are able to employ EFs to discover differences in ASD-implicated biological processes. FGF stimulates proliferation and likely maintains a NSC-like state. In comparison of an ASD-Sib pair, this experimental paradigm has begun to uncover patient-specific differences in cellular phenotypes, as might be expected in idiopathic ASD.

Disclosures: **M. Williams:** None. **S. Prem:** None. **X. Zhou:** None. **P. Matteson:** None. **P. Yeung:** None. **C. Lu:** None. **Z. Pang:** None. **L. Brzustowicz:** None. **J. Millonig:** None. **E. DiCicco-Bloom:** None.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Topic: C.06. Developmental Disorders

Support: New Jersey Governor's Council for Medical Research and Treatment of Autism (PI: Millonig)

New Jersey Governor's Council for Medical Research and Treatment of Autism CAUT14APL031 (PI: DiCicco-Bloom)

Title: Autism patient derived neural stem cells (nscs) exhibit neurite extension and migration phenotypes

Authors: *S. PREM[1], M. WILLIAMS[1], X. ZHOU[1], P. MATTESON[1], P. YEUNG[2], C. LU[2], Z. PANG[2], L. BRZUSTOWICZ[3], J. MILLONIG[1], E. DICICCO-BLOOM[1];
[1]Rutgers, Robert Wood Johnson, Piscataway, NJ; [2]Rutgers, Robert Wood Johnson, Child Hlth. Inst., New Brunswick, NJ; [3]Div. of Life Sci., Rutgers Univ., Piscataway, NJ

Abstract: Autism spectrum disorders (ASD) are developmental disorders defined by abnormal social interactions and repetitive behaviors. Elucidation of ASD etiology has been hampered by inability to study human neurons, disorder heterogeneity and relevance of model systems. Recently, induced pluripotent stem cell (iPSC) studies in monogenic autism and sporadic schizophrenia uncovered abnormalities in synaptic function and neuronal migration in human neurons. To examine deficits in sporadic autism, we (NJ Autism Center of Excellence; PI Millonig) generated iPSC lines from 8 severely affected males with ASD and their unaffected brothers (Sib) and derived NSCs for study. We are comparing neurite extension and cell migration in ASD and Sib lines in control media and media with relevant growth factors such as PACAP, serotonin and neurotrophins. Growth factor stimulation of signaling pathways may allow identification of defects in idiopathic autism. To quantify neurite outgrowth, confluent NSCs were dissociated and plated on fibronectin coated plates in control or PACAP (10nM) media at low density. At 48h, cells bearing neurites >2 cell diameters were counted in 10 fields. To define migration, neurospheres were formed by plating NSCs in absence of substrate. After 48-96h, spheres were plated on Matrigel with control or PACAP media and fixed at 48h. Using phase contrast images, the areas of the inner cell mass and total sphere outgrowth were measured. Migration: total neurosphere area-inner cell mass area. In one Sib, 13% of cells had neurites in control media while 21% had neurites in PACAP reflecting a 58% increase ($p=0.007$). However in ASD, only 7.3% of cells had neurites in control, a significant reduction compared to their Sib ($p=0.001$). Further, PACAP elicited no change in neurite outgrowth in ASD (PACAP; 7.4%). Similarly, Sib neurosphere migration was increased 77% by PACAP (Con=1318; PACAP=2107; $p<0.001$), whereas ASD exhibited reduced migration compared to their Sib ($p=0.001$) and no PACAP response (Con= 855; PACAP=962, $p=0.15$). While ASD NSCs display absent neurite outgrowth with PACAP, preliminary studies suggest other factors overcome outgrowth deficiency, suggesting pathway specific deficits in this individual. Thus, baseline and PACAP induced migration and neurite extension are significantly reduced in ASD NSCs in one family. Characterization of other sibpairs will determine if these results are generalizable. While heterogeneity of ASD reduces the chances of identical phenotypes, our studies show the value of utilizing relevant growth factors to uncover impaired and patient specific developmental pathways which may lead to personalized ASD therapies.

Disclosures: **S. Prem:** None. **M. Williams:** None. **X. Zhou:** None. **P. Matteson:** None. **P. Yeung:** None. **C. Lu:** None. **Z. Pang:** None. **L. Brzustowicz:** None. **J. Millonig:** None. **E. DiCicco-Bloom:** None.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Title: Testosterone and the Brain: A molecular study into Autism Spectrum Disorders using a human stem cell model

Authors: *D. ADHYA[1], K. JOZWIK[2], J. CARROLL[2], J. PRICE[3], D. P. SRIVASTAVA[3], S. BARON-COHEN[1];
[1]Psychiatry, [2]Univ. of Cambridge, Cambridge, United Kingdom; [3]Inst. of Psychiatry, King's College, London, London, United Kingdom

Abstract: Hans Asperger first described autism in 1944 as an extreme variant of male intelligence. Individuals with this condition show difficulty in empathising - in making sense of and predicting another's feelings, thoughts and behaviour, and excel in systemising - a preference for rule-based, structured, factual information. Studies have shown this extreme variant of male intelligence in autism spectrum disorders (ASD) to be correlated with elevated foetal steroidogenic activity, suggesting a role for steroids such as testosterone in the development of the ASD pathology during foetal development. The purpose of this study was to determine the effect of testosterone on cellular and molecular pathways in the ASD brain, and how it differs from the typically developing (TD) brain. This was achieved using a human induced pluripotent stem cell (hiPSC) model of this condition. Keratinocytes from healthy individuals, and individuals diagnosed with ASD were reprogrammed into iPSCs, then differentiated into cortical neurons using the dual SMAD signalling inhibition strategy. Testosterone was administered to these neurons at an early stage of development at the physiological and supra-physiological levels. Preliminary data indicates that ASD neurons seem to develop precociously, while being more responsive to testosterone than healthy neurons. The androgen receptor (AR) and some of its putative downstream genes such as gonadotropin-releasing hormone (GnRH) and p38 (a class of mitogen-activated protein kinases) were

differentially expressed in ASD neurons compared to TD neurons, predicting differential structural and functional outcomes of neurons in the two groups. Genes indicating neuron cell fates such as T-box brain 1 (TBR1) and BRN2 were also differentially expressed in ASD neurons, demonstrating altered characteristics of developing ASD neurons. These data suggests that the ASD phenotype starts developing *in utero* at very early stages of brain development, and that neurons of ASD individuals are programmed, at a molecular level, to react differently to sex steroids such as testosterone.

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Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Topic: C.06. Developmental Disorders

Support: Helmholtz Gesellschaft VH-VI-510

European Unions Seventh Framework Programme FP7/2007-2013

Title: Characterization of zinc biology in the Autism Spectrum associated Disorder Phelan McDermid Syndrome using patient biosamples and iPS derived cells

Authors: *S. PFAENDER[1], A. GRABRUCKER[2], T. BOECKERS[1];
[1]Inst. for Anat. and Cell Biol., Ulm Univ., Ulm, Germany; [2]Mol. Analysis of Synaptopathies, Neurocenter, Ulm University, Germany

Abstract: Phelan McDermid Syndrome (PMDS) is a neurodevelopmental disease characterized by infantile hypotonia, developmental delay, impaired or absent speech, seizures and features of Autism Spectrum Disorders (ASD). The major cause for the observed phenotype is a 22q13.3 deletion involving the SH3 domain and ankyrin repeat containing protein 3 (Shank3), a scaffolding protein that forms highly organized multimeric platforms at the postsynaptic density (PSD) of excitatory synapses upon zinc-binding. Since zinc deficiency has been reported repeatedly in ASDs, here we examined a cohort of PMDS patients for zinc levels in blood and hair along with the assessment of a range of symptoms. We found an increased incidence rate of zinc deficiency, which is associated with the occurrence of certain symptoms. To understand the possible reasons for zinc deficiency in this patient group, we screened the expression of zinc and

other metal homeostasis proteins in enterocytes differentiated from human induced pluripotent stem (iPS) cells from both PMDS patients and healthy controls. Our results indicate an impaired cellular zinc absorption in PMDS patients. Since recruitment and multimerization of soluble Shank3 at the PSD are zinc dependent, disruption of zinc homeostasis in neurons might alter neuronal development and function in various ways. For example, given that zinc plays a major role in the function of several proteins involved in proliferation and apoptosis of neuronal precursors as well as neuronal development and function, we also examined expression of zinc transporters in differentiated and differentiating neurons of iPS cells. Since we are particularly interested in hypotonia and motor development, we chose motor neurons for our model system. Here, we analyzed zinc homeostasis in different stages of neuronal differentiation and examined the effects of zinc deficiency on both morphology and function of motor neurons differentiated from iPS cells from PMDS patients and healthy controls. Furthermore, we evaluated whether alterations in PMDS motor neurons can be rescued by targeted manipulation of biometal homeostasis as novel treatment strategy for PMDS.

Disclosures: **S. Pfaender:** None. **A. Grabrucker:** None. **T. Boeckers:** None.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.16/F4

Topic: C.06. Developmental Disorders

Support: Ministry of Education, Science, Technology, Sports and Culture of Japan 23590124

Title: Comprehensive approach with an analytical battery to elucidate pathophysiological role of RBFOX1/A2BP1, a "hub" gene in the ASD gene transcriptome network

Authors: *K.-I. NAGATA[1], N. HAMADA[2], H. ITO[2], H. TABATA[2];
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Abstract: While many different biological causes have been implicated in the etiologies of neurodevelopmental disorders such as autism-spectrum disorder (ASD) and intellectual disability (ID), genetic factors are considered to be the most important. Thus, it is essential to clarify the pathophysiological significance of respective disease-related genes in brain development. To address this issue, we have established a battery of *in utero* electroporation-based *ex vivo* and *in vitro* observations including cortical neuron migration, axon elongation, dendrite development,

spine morphogenesis and live-imaging as well as cell biological and biochemical analyses. RBFOX1/A2BP1 is a neuron-specific splicing factor predicted to regulate neuronal splicing networks clinically implicated in neurodevelopmental disorders including ASD. Since RBFOX1 has been recently identified as a "hub" in the ASD gene transcriptome network, we performed comprehensive analyses of RBFOX1 by with the above-mentioned analytical battery. Knockdown of RBFOX1 using *in utero* electroporation caused abnormal cortical neuron positioning during corticogenesis, which was most likely to be attributed to impaired cell migration. Based on the confocal laser microscope-associated live-imaging analyses, migration defects were found to occur in the radial migration and terminal translocation, perhaps due to impaired nucleokinesis. Notably, RBFOX1-deficient neurons frequently showed abnormal polarity during corticogenesis. On the other hand, the cell cycle of neuronal progenitor cells was not affected by RBFOX1-silencing. RBFOX1 was also found to regulate axon/dendrite development since axon extension to the opposite hemisphere as well as dendritic arborization was suppressed in RBFOX1-deficient cortical neurons. Effects of RBFOX1 knockdown on the neuronal morphology were further confirmed in *in vitro* analyses. RBFOX1-silencing in primary cultured hippocampal neurons resulted in the reduction of primary axon length and total length of dendrites, and abnormal spine morphogenesis. Taken together, impaired cortical neuron migration, disturbed interhemispheric axon development and dendritic arborization, and defective synapse formation may induce structural and functional defects of the cerebral cortex, and consequently contribute to emergence of the clinical symptoms of neurodevelopmental disorders.

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Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Topic: C.06. Developmental Disorders

Support: NIH Grant R01DA035263

Title: Zn2+ reverses functional deficits in a de novo dopamine transporter variant associated with autism spectrum disorder

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Abstract: Our laboratory recently characterized a novel autism spectrum disorder (ASD)-associated de novo missense mutation in the human dopamine transporter (hDAT) gene SLC6A3 (hDAT T356M). This hDAT variant exhibits dysfunctional forward and reverse transport properties that may contribute to DA dysfunction in ASD. Here, we report that Zn2+ reverses, at least in part, the functional deficits of ASD-associated hDAT variant T356M. These data suggest that the molecular mechanism targeted by Zn2+ to restore partial function in hDAT T356M may be a novel therapeutic target to rescue functional deficits in hDAT variants associated with ASD.

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Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.18/F6

Topic: C.06. Developmental Disorders

Title: Effects of trace metal imbalances on excitatory synapses of hippocampal neurons

Authors: *S. HAGMEYER[1], J. CRISTÓVÃO[2], K. MANGUS[1], C. M. GOMES[2], A. M. GRABRUCKER[1];
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Abstract: Recent studies revealed that biometal dyshomeostasis plays a crucial role in the pathogenesis of neurological disorders such as autism spectrum disorders (ASD). This deregulation can be caused either by environmental factors such as diet, malabsorption of essential trace metals, exposure to putative toxic metals, infection, stress, or by pathological abnormalities in the brain such as the accumulation of metal binding proteins like Amyloid beta (Aβ) or S100B. Disrupted neuronal trace metal homeostasis in turn may mediate synaptic dysfunction and impair synapse formation and maturation. Here, we investigated the consequences of an imbalance of transition metals on glutamatergic synapses of hippocampal neurons *in vitro*. We analyzed whether an imbalance of any one metal ion alters synapse

formation and subsequently established a biometal profile in the culture medium that mimicked the characteristic changes in trace metals reported in ASD patients. Furthermore, we manipulated trace metal levels by the exposure of neurons to calcium and zinc binding S100B protein aggregates and/or copper and zinc binding Aβ aggregates. To investigate the impact of these alterations on synapses, we evaluated synapse formation and maturation, and composition regarding NMDA receptor subunits and Shank proteins. Our results show that a biometal profile characteristic for ASD patients leads to a reduction of NMDAR subunit 1 and 2a levels as well as synaptic Shank protein family members (Shank2, Shank3) along with a reduction of synapse density. Moreover, exposure to putative toxic protein aggregates leads to trace metal imbalances and similarly affects synapses and synaptic proteins supporting the hypothesis that a part of the pathology of these aggregating proteins is a consequence of altered synaptic metal homeostasis. Thus, we conclude that balancing trace metal levels in synaptopathies such as ASD and Alzheimer's Disease might be a prime target to normalize synaptic alterations.

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Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Topic: C.06. Developmental Disorders

Support: NARSAD 21180

NIMH MH087879

NIMH MH089176

Title: Integrative analysis of RNA-seq and whole genome sequencing reveals perturbed gene co-expression networks in autism

Authors: *G. COPPOLA[1], K. ARDHANAREESWARAN[1], J. MARIANI[1], A. ABYZOV[2], F. VACCARINO[1];
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Abstract: In our recently published work (Mariani et al, 2015) we exploited iPSCs-derived telencephalic organoids to model idiopathic ASD. We analyzed the transcriptome of the

organoids from patients (PB) and their unaffected fathers (NC). ASD-derived organoids demonstrate an accelerated cell cycle and an overproduction of GABAergic inhibitory neurons and synapses. Transcription factor (TF) analysis highlighted the Forkhead TF family as possible driver of gene upregulation and FOXG1 was among the top upregulated genes in ASD-derived neurons. Using RNA interference, we show that by normalizing the expression of FOXG1 we recover the GABA/glutamate balance. We therefore identified in the strong up-regulation of FOXG1, a key driver of the GABA/glutamate imbalance. We performed whole genome sequencing (WGS) in the same set of PBs and identified rare SNVs in regulatory regions associated to FOXG1, albeit not in every PB. We then extended the analysis to FOXG1 centric gene co-expression networks (GCN). We identified: 1) a set of genes strongly correlated to FOXG1 in NC and weakly correlated in PB, i.e. loss-of-correlation network (LCN); 2) a set of genes showing weakly correlated to FOXG1 in NC and strong correlation in PB, i.e. gain-of-correlation network (GCN). The LCN contains several genes crucial for glutamatergic differentiation. The GCN contains several genes crucial for GABAergic differentiation. This is consistent with our recently published work, and reflects the identified GABA/glutamate imbalance in probands, strengthening the role of FOXG1 as key driver of this phenotype. We used FunSeq2 to functionally associate SNVs to genes, score them for their potential disruptive impact and filter out any variant with a score < 1.5. We identified 205 genes recurrent in all 4 probands. Functional annotation highlighted splicing, synaptic transmission and immune system. We tested the FOXG1 centric networks for enrichment in the entire set of gene involved by the 4 probands and found significant overlap with the LCN (p-value=$6.78\ 10^{-8}$). Finally, to validate our findings, we performed WGS of four more patients, infer the set of rare SNVs and tested them again for convergence and overlap with the FOXG1-centric networks. We found 219 genes recurrent in all 4 probands. Functional annotation highlighted a strong involvement of immune system related gene. Again, we found significant overlap with the LCN (p-value=$1.56\ 10^{-15}$). Our integrative analysis strengthens our previous finding of the involvement of FOXG1, strongly involving its potential regulatory network and highlights the importance of the non-coding regions in disease etiology.

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Poster

679. Autism: Synaptic and Cellular Mechanisms II

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: NYU Challenge Grant

Title: A neuroenergetic model of the autism brain predicts clinical results from postmortem donors

Authors: *Z. SACCOMANO[1], E. C. AZMITIA[2];
[1]Biol. and Psychology, New York Univ., New York, NY; [2]Biology, Psychiatry, Ctr. for Neural Sci., New York Univ., New York City, NY

Abstract: Autism spectrum disorders are diagnosed by clinical assessments aimed to identify individuals with an abnormal behavioral phenotype marked by social and communication deficits as well as stereotyped or repetitive rituals. Autism is also associated with a range of cellular abnormalities such as accelerated perinatal brain growth (Shen et al., 2013; Courchesne et al., 2011), glial hyperactivation and inflammation (Vargas et al., 2005; Tetreault N.A. et al., 2012), increased electrical activity (Rumsey and Ernst, 2000), brain hypoperfusion (Burroni et al., 2008; Boddaert et al., 2002), and persistent neurovascular reorganization (Azmitia et al., 2015). Furthermore, some evidence suggests situations that increase energy flow into the brain may promote normative behaviors in autism patients through hyperbaric oxygen therapy (Rossignol et al., 2006) and, interestingly, when patients experience fevers, which increases cerebral blood flow (Good, 2011). These results indicate that changes in neuroenergetic maintenance maybe more central to generating autistic behaviors than altered morphology. Here, we present a model of energy propagation amongst vascular cells, astrocytes and neurons in the autism brain by measuring the integrity of their interfaces in human postmortem samples. Morphometric analyses of these networks were conducted in human postmortem tissue and we demonstrate that the integrity of the neurovascular unit in superior temporal cortex (layers II-VI) predicts clinical results specific to that region from the postmortem brain donors. Currently, developing models of this kind is the only method capable of correlating altered cellular networking through direct microscopy in human postmortem tissue with dynamic brain activity measured in living patients.

Disclosures: **Z. Saccomano:** 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.; NYU Challenge Grant. **E.C. Azmitia:** 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.; NYU Challenge Grant.

Poster

679. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 679.21/F9

Topic: C.06. Developmental Disorders

Title: Annotation and curation of autism-related protein-protein interaction datasets into AutDB, a genetic database for autism

Authors: *W. PEREANU, C. CROFT SWANWICK, S. MUEND, S. BANERJEE-BASU; Mindspec, Mc Lean, VA

Abstract: Research over the last few decades has linked hundreds of genes with diverse roles to autism spectrum disorder (ASD). The autism-related genes encode proteins with roles that include ion channels, cell adhesion molecules, chromatin remodelers, transcription factors, RNA binding proteins and many others. Understanding how such a large and diverse set of genes contribute to ASD will require a bioinformatics approach and a well annotated dataset sourced from the works of multiple research groups. To this end, we have developed an ASD-specific database (PIN) that integrates evidence of protein-protein interactions from publish papers. The PIN database includes all high-priority ASD-linked genes as scored by the Gene Scoring module of SFARI Gene (https://gene.sfari.org/autdb/GS_Home.do). Specifically, we annotate all ASD-linked genes that are scored as high confidence (category 1), strong evidence (category 2), syndromic and genes that have both functional and genetic evidence (Category 3). Because of the diverse protein roles that ASD-linked genes take part in, we have developed an inclusive ontology that captures the experimental techniques used and the tissue types tested. We also categorize the type of protein interaction using a set of six terms that cover physical binding and functional regulation, these are “Protein Binding”, “DNA Binding”, “RNA Binding”, “Protein Modification” and “Autoregulation”. The PIN database is updated on a quarterly basis (December, March, June and September). As of the March, 2015 release, the PIN database includes 339 ASD-linked genes that have 30,780 interactions from 29 species that have been sourced from 2,274 primary research articles. The PIN database has been integrated into our genetic autism database, AutDB and can be found here: http://autism.mindspec.org/autdb/PINHome.do. In addition, we have licensed the database and it may also be accessed through SFARI Gene (https://gene.sfari.org/autdb/PINHome.do).

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Poster

680. Autism: Physiology and Systems

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 680.01/F10

Topic: C.06. Developmental Disorders

Title: The neural substrate of autism spectrum disorder and attention hyperactivity disorder

Authors: *H. OHTA[1], T. ITAHASHI[1], C. KANAI[1], M. NAKAMURA[3,1], K. KANJI[1], H. YAMADA[2], A. IWANAMI[2], N. KATO[1], R. HASHIMOTO[1,4];
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Abstract: [Introduction] Autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) are neurodevelopmental disorders. Previous researches have shown high rate of ADHD comorbidity (30-50%) in individuals with ASD. According to the Diagnostic and Statistical Manual of Mental Disorders_5th edition (DSM-5), ASD and ADHD can be diagnosed together. There are, however, only a few studies investigate the commonality and/or difference of biological characteristics between the two disorders. Structural and functional magnetic resonance imaging (MRI) has shown brain abnormalities in ASD and ADHD. Recent neuroimaging technique makes it possible to investigate white matter fiber structure using Diffusion Tenor Imaging (DTI). Not only the localized brain abnormality, but impaired structural brain connectivity are considered to be related the core feature of ASD and ADHD. So far, there is no study that compared the two disorders regarding the characteristics of white matter tract. The aim of the present study is to reveal the commonality and/or difference of white matter microstructure between the two disorders. [Method] Twelve adults with ASD, five adults with ADHD and eight age- and gender- matched healthy controls participated in this study. Individuals who have mental retardation were excluded from this study. MRI scans were conducted to all participants. The DTI data were compared between groups. [Result] Disorder-specific white matter alternations were found in some regions. However, the differences between groups were not significant statistically ($p > 0.05$). [Conclusion] Due to the small sample size, significant difference was not found between groups. This is on going study, so we will recruit more participants to reveal the commonality and/or difference of white matter microstructure between the two disorders.

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Poster

680. Autism: Physiology and Systems

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Topic: C.06. Developmental Disorders

Support: The National Natural Science Foundation of China (81271507)

The Research Special Fund for Public Welfare Industry of Health of China (201302002-11)

Title: An mri study in autistic children:structural and functional abnormalities in brain areas related to central oxytocin and arginine-vasopressin system

Authors: *X.-J. SHOU[1,2,4], J.-S. HAN[1,2,4], R. ZHANG[1,2,4], X.-J. XU[1,2,4], X.-Z. ZENG[5], Y. LIU[5], H.-S. YUAN[5], Y. XING[6], M.-X. JIA[7,8], Q.-Y. WEI[9], S.-P. HAN[3];
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Abstract: Autism is a developmental disorder with characteristic deficiency in social behavior and communication skills and the presence of stereotypical behavior. Several lines of evidence suggest that oxytocin (OXT) and arginine-vasopressin (AVP) system were aberrant in the autistic patients, and administration of exogenous OXT and AVP could partially and temporarily relief the clinical symptoms. Our previous studies also demonstrated that transcutaneous electrical acupoint stimulation (TEAS) improved autistic behaviors accompanied by changes of plasma levels of OXT/AVP. Certain brain regions such as hypothalamus, amygdala and hippocampus are known to contain OXT/AVP neurons/terminals and play important roles in regulation of complex social behaviors. Thus, we designed a clinical study to investigate the correlations between the autistic symptoms, circulating OXT/AVP levels and brain structural and functional abnormalities as assessed by MRI techniques. The results showed: (1) the volume of hypothalamus was decreased, and the left amygdala and left hippocampus increased in children with autism compared to typically developing children. (2) The functional connectivity between amygdala (AMG) and supramarginal gyrus (SMG) was decreased in children with autism compared to typically developing children. An attenuation of the functional connectivity between AMG and SMG was negatively correlated with increased clinical Autism Behavior Checklist (ABC) scores (total and sensory scores). (3) The functional connectivity between the left

hippocampus and right basal ganglia/right thalamus areas was increased in children with autism compared to the typically developing children. (4) A lowering of the blood OXT/AVP concentration was positively correlated with a decrease of the connectivity between AMG and ISMG. These results strongly suggest that changes in structure and activity of intrinsic OXT/AVP system may be involved in the development of autism.

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Poster

680. Autism: Physiology and Systems

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Topic: C.06. Developmental Disorders

Support: RS Macdonald Charitable Trust

Patrick Wilde Centre

The University of Edinburgh

The Scottish Mental Health Research Network

Title: Investigation of *in vivo* glutamate concentrations in autism spectrum disorders with single-voxel spectroscopy

Authors: *J. E. SIEGEL-RAMSAY[1], S. ELEY[1], S. CAMPBELL[2], H. BRANIGAN[3], A. STANFIELD[1], M. DAUVERMANN[4], S. LAWRIE[1];
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Abstract: Several studies have linked glutamate alterations to autism spectrum disorder (ASD). Magnetic resonance spectroscopy (MRS) is a useful technique to non-invasively measure glutamate concentrations in-vivo. To our knowledge, this is the first study to apply MRS to the measurement of glutamate concentrations in adults with high-functioning ASD in the dorsal anterior cingulate cortex (dACC), a region high in glutamatergic signalling and previously shown to function abnormally in participants with ASD. We hypothesized that glutamate concentrations, as measured by single-voxel spectroscopy (SVS), would be altered in the dACC

in participants with ASD and would correlate with autistic symptoms as measured by the Autism Diagnostic Observation Schedule (ADOS). All participants underwent neuropsychological testing, structural Magnetic Resonance Imaging and SVS. Structural scans were analysed with whole brain voxel based morphometry (VBM). We used a SVS protocol that was designed for the optimised assessment of the glutamate peak. Additional metabolite measurements included N-acetylaspartate (NAA), glutamate/glutamine (Glx), creatine, phosphocholine and myo-inositol. Lastly, we ran bivariate Pearson correlations between glutamate concentrations, demographic (age, IQ) and clinical scores (ADOS). SVS was acquired in 19 participants with ASD (ADOS > 6) and 19 neurotypical controls (NC). There were no significant differences between groups for age, IQ, tissue composition in the dACC or whole brain grey matter volume as measured by VBM. Participants with ASD had, on average, a non-significant reduction in glutamate and Glx concentrations compared to NC. NAA, an indicator of neuronal function, was significantly reduced in participants with ASD ($p<0.05$). There was no support for the inter-relationship between glutamate concentrations and the ADOS in participants with ASD. We conducted a second analysis which excluded four participants with ASD due to factors which may have confounded glutamate concentrations (three participants on anticonvulsant medications and one participant with a reported history of seizures). The second analysis replicated all of the findings from the full group analysis, except for a significant reduction in glutamate ($p<0.05$) and Glx ($p<0.05$) concentrations for participants with ASD relative to NC. In conclusion, we provide evidence of altered glutamate and NAA concentrations in adults with ASD. Glutamate may be a potential target for therapeutic intervention but further investigation is required to gain insight into how glutamate is linked to both the pathophysiology and behavioural manifestations of ASD.

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Poster

680. Autism: Physiology and Systems

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Topic: C.06. Developmental Disorders

Support: NIH Grant R01DC011339

Title: Transdiagnostic neural basis for impaired phonological working memory across reading disability and autism spectrum disorder

Authors: *Z. QI[1], C. LU[2], A. HARRIS[3], L. W. WEIL[3], M. HAN[1], K. HALVERSON[1], T. K. PERRACHIONE[3], M. KJELGAARD[1], W. KENNETH[1], H. TAGER-FLUSBERG[3], J. D. E. GABRIELI[1];
[1]MIT, Cambridge, MA; [2]Beijing Normal Univ., Beijing, China; [3]Boston Univ., Boston, MA

Abstract: Individuals with reading disability or autism spectrum disorder (ASD) have, respectively, distinct disorders of language and social communication, but often exhibit common impairment in phonological abilities. It is unknown, however, whether the impaired phonological abilities reflect distinct or shared neuroanatomical bases in these two diagnostic groups. Here, we examined white-matter structural connectivity via diffusion-weighted imaging in children with reading disability, or ASD, or typical development (TD) matched in age, non-verbal IQ and gender. Phonological working memory is assessed by a combination of phonological awareness and verbal short-term memory tasks. Compared with TD, both reading disability and ASD groups exhibited reduced phonological working memory. Both groups displayed lower fractional anisotropy and higher radial diffusivity in the temporo-parietal part of the left arcuate fasciculus (TP-AF). ASD group additionally exhibited white-matter anomalies at the temporo-occipital part of the right inferior longitudinal fasciculus (TO-ILF). No group difference was found between reading disability and ASD. The fractional anisotropy of the left AF and right ILF correlated with ability in phonological working memory across all participants. In particular the microstructural features of the right ILF only correlated with phonological working memory in the two diagnostic groups, but not the typically developing counterparts. Thus, impaired phonological working memory was transdiagnostically associated with a similar neuroanatomical endophenotype.

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Poster

680. Autism: Physiology and Systems

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Topic: C.06. Developmental Disorders

Support: NIMH 1R01 MH081023

K01 MH097972

Title: Local resting state functional connectivity in autism: Multisite variability and effect of eye status

Authors: *S. NAIR[1], M. M. BERKEBILE[1], C. P. CHEN[1], J. O. MAXIMO[2], R. A. MÜLLER[1,3]; [1]San Diego State Univ., San Diego, CA; [2]Univ. of Alabama, Birmingham, Birmingham, AL; [3]UCSD, San Diego, CA

Abstract: Despite growing evidence of abnormal long distance connectivity in autism spectrum disorder (ASD), findings for local connectivity have been limited and inconsistent. A regional homogeneity (ReHo) study by Maximo et al. (2013) reported local overconnectivity in posterior visual cortices in ASD. This was not confirmed in a large-sample study by the Autism Brain Imaging Data Exchange (ABIDE; DiMartino et al., 2013). However, eye status (open vs. closed) was not considered in these studies. Using high-quality data from ABIDE and our own group, we aimed to resolve these inconsistencies. Resting state fMRI data from ABIDE and SDSU (in-house) were processed. Time points with motion >.2mm were censored, and participants with <80% time points remaining after censoring were excluded. Nuisance regressors included six rigid-body motion parameters, signal from white matter and ventricles, and derivatives. Analyses were performed with and without global signal regression (GSR). Voxel-wise ReHo (local connectivity) maps were obtained using AFNI's 3dReHo. Five separate, low motion datasets were analyzed: a Grand Total group including all data (ABIDE and SDSU combined; N=331), SDSU (eyes open [EO]; N=53), NYU (eyes open; N=63), ABIDE-EO (eyes open, excluding SDSU and NYU; N=141), and ABIDE-EC (eyes closed; N=72). All subsamples were matched on head motion (p>.96), age (p>.87), and nonverbal IQ (p>.96). Despite large high-quality subsamples, between-group effects differed across datasets. Only a single effect, ASD overconnectivity in visual cortex, was seen across 4 datasets (Grand Total, SDSU, NYU, ABIDE-EO). Consistent effects for ≥2 datasets included underconnectivity in pericentral and inferior premotor regions (Grand Total, SDSU, ABIDE-EC), in mid/posterior cingulate cortex (Grand Total, SDSU), and in medial prefrontal cortex (Grand Total, NYU). Results from the non-GSR pipeline were largely similar. Local overconnectivity in visual cortex was the most consistent finding across multiple datasets, including a Grand Total with N=331. The finding is in agreement with previous reports (Maximo et al., 2013; Keown et al., 2013) and may relate to atypically increased visual activity often seen in the ASD imaging literature. The divergent finding in DiMartino et al. (2013) can be attributed to inclusion of eyes-closed data - the only dataset not showing this finding. Despite several other partly consistent findings, the differences across datasets suggest that ReHo may be highly sensitive to between-site variability, even in high-quality low-motion datasets.

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Poster

680. Autism: Physiology and Systems

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Program#/Poster#: 680.06/F15

Topic: C.06. Developmental Disorders

Title: Abnormal cerebellar functional connectivity in children with autism spectrum disorder

Authors: *R. HANAIE[1], I. MOHRI[1], K. KAGITANI-SHIMONO[1], I. HIRATA[2], J. MATSUZAKI[1], F. NAGATANI[1], Y. WATANABE[3], M. TANIIKE[1];
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Abstract: The cerebellum is one of the brain regions that has been most consistently reported to have neuropathological findings in patients with autism spectrum disorder (ASD). In recent years, many studies have reported that the cerebellum contributes not only to motor function but also to various cognitive functions. Aim of this study was to investigate cerebellar functional connectivity in children with ASD using resting state functional magnetic resonance imaging and to know whether its abnormalities were related to motor deficits, socio-communicative deficits, and executive dysfunction in children with ASD. Echo planar imaging functional volume and T1-weighted images of 15 children diagnosed with ASD (all males; age: 11.1 ± 2.1 years) and 20 typically developing (TD) children (19 males/1 female; age: 10.7 ± 2.4 years) were acquired on a 3 tesla scanner. The diagnosis of ASD was made according to the DSM-IV criteria and confirmed by autism diagnostic schedule-generic (ADOS-G). The Movement Assessment Battery for Children 2 (M-ABC 2) was used to assess motor function of the participants. In addition, Social Responsiveness Scale (SRS) and Behavior Rating Inventory of Executive Function (BRIEF) were used to assess socio-communicative function and executive function, respectively. Seed-based resting state functional connectivity analysis was performed using the CONN toolbox (http://www.nitrc.Org/projects/conn/). Cerebellar ROIs were created using the probabilistic MR Atlas of the human cerebellum (Diedrichsen, 2009). Children with ASD showed overall decreased cerebellar connectivity relative to TD children. The decreased functional connectivities between several cerebellar subregions and brainstem, thalamus, insula, prefrontal, premotor, and parietal cortex were found. In the ASD group, connectivity between the left dentate nucleus and brainstem was positively related to the total score of the M-ABC 2, and connectivity between the left VIIIa and left thalamus was positively related to the Restricted/Repetitive Behavior (RRB) score of the SRS. In addition, connectivity between the

right VIIb and right insula was negatively related to the Behavioral Regulation Index (BRI) score of the BRIEF. This study clearly showed that there were abnormalities in connectivity between cerebellum and various other brain regions, and abnormal cerebellar connectivity contribute to motor, executive and socio-communicative deficits in children with ASD.

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Poster

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Autism Science Foundation Grant 12-1001

Title: Atypical intrinsic functional connectivity of FFA is associated with social communication deficit in ASD

Authors: *W. ZHAO, I. FISHMAN, A. LEGENKAYA, S. NAIR, M. SULLIVAN, Y. GAO, M. BERKEBILE, R.-A. MUELLER;
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Abstract: Among the core symptoms of Autism Spectrum Disorder (ASD) are deficits in social communication. There is large body of evidence demonstrating that individuals with ASD have impairments in face processing, a crucial component of reciprocal social interactions, including deficits in gaze processing, face identification and recognition of facial expressions of emotion. Fusiform face area (FFA), located in the ventral occipital cortex, is essential for face perception and has been shown to have reduced activation to faces in individuals with ASD compared to typically developing (TD) controls. Given the prevalent theory that ASD is a disorder of network dysfunction and abnormal brain connectivity, we investigated functional network organization of FFA in individuals with ASD, using intrinsic functional connectivity (iFC), a functional imaging method assessing the synchronicity of spontaneous low-frequency activity fluctuations between

different brain areas. We performed whole-brain iFC analysis using right FFA as a seed region in 35 children and adolescents with ASD (ages 8-17 years) and 36 TD participants matched for age, gender, IQ, and in-scanner head motion. Direct group comparisons revealed that the ASD group showed two clusters of greater connectivity (compared to the TD group): with a region in the inferior portion of the medial frontal gyri (medial frontopolar cortex) and with posterior cingulate cortex (PCC). Reduced connectivity in ASD was observed between FFA and the right supplementary motor area (SMA). Within the ASD group, the degree of overconnectivity (mean z-score) between FFA and the medial frontal cluster was significantly correlated with social impairment, as measured by the Social Responsiveness Scale (SRS), i.e., participants with greater sociocommunicative symptoms had more excessive connections. The findings show atypical iFC patterns of a brain region crucially involved in face processing in children and adolescents with ASD. Namely, greater connectivity was observed between FFA and regions outside of the canonical face processing network (e.g., PCC, medial frontopolar cortex), suggesting atypical network organization of the neural circuit underlying face perception in ASD. The link between excessive out-of-network functional connectivity of the FFA and ASD social communication symptoms suggests that inefficient face processing circuitry may contribute to social deficits observed in ASD. Consistent with a previous study (Fishman et al., JAMA Psychiatry 2014), the finding suggests that autistic symptomatology may be tied to functional overconnectivity.

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Poster

680. Autism: Physiology and Systems

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Program#/Poster#: 680.08/F17

Topic: C.06. Developmental Disorders

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Title: Delineating adaptive neural mechanisms in individuals with autism when processing unimodal vs. bimodal real-world stimuli using functional MRI

Authors: ***P. J. WEBSTER**[1], C. FRUM[2], A. KURKOWSKI-BURT[3], J. W. LEWIS[1]; [1]Neurobio. & Anat., [2]Physiol. and Pharmacol., [3]Occup. Therapy, West Virginia Univ., Morgantown, WV

Abstract: The rate of autism diagnosis has been steadily increasing in the U.S. according to the Centers for Disease Control. Sensory processing dysfunction is a core feature of autism that can cause an individual to over-respond or under-respond to everyday sensory stimuli. The inability to effectively manage non-noxious stimuli can be socially isolating, increase anxiety, and impact a person's ability to attend to what's pertinent in their environment. Neuroimaging studies investigating sensory processing in autism have shown differential structural and functional connectivity depending on the age of the participants, level of stimulus complexity, and task demands. In addition, the timeframe within which information is perceived is altered in individuals with autism. Despite these differences--and in spite of the fact that cortical activation patterns in this group may be seen in different brain regions and with greater variability--autistic children and adults often perform at or above the level of their peers on many tasks. Exactly how individuals with a spectrum of autism phenotypes develop adaptive neuronal mechanisms to process and integrate sensory information has not been determined. The objective of this research is to depict cortical processing systems engaged during a simple selective attention task using real world (complex) audiovisual stimuli in high-functioning individuals with autism. We hypothesize that differences in structural and functional connectivity induce adaptive neural mechanisms that impact the timing of incoming sensory inputs and alter neuronal processing when integrating sensory information. We used functional magnetic resonance imaging (fMRI) to characterize these putative adaptive brain mechanisms when processing one source of sensory input (visual) vs. integrating two types of sensory information (audiovisual). The preliminary data indicate that adolescents and young adults with autism show differential activation in the cingulate region of the brain when integrating audiovisual information compared to adolescents and adults without autism. Differences in activation in this brain region may contribute to the high comorbidity of ADHD in individuals with autism as well as in emotional dysregulation, often seen in autism. This research is significant because it utilizes real world stimuli to investigate cortical activation differences across two developmental time points (adolescents and young adults), thus will inform models of plasticity in this heterogeneous disorder and will aid in the refinement of targeted interventions and their efficacy.

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Poster

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Topic: C.06. Developmental Disorders

Title: Hyper-synchronization of brain activity in ASD during face-to-face conversation

Authors: *K. JASMIN[1,2], S. J. GOTTS[2], Y. XU[3], S. LIU[3], C. RIDDELL[2], J. INGEHOLM[2], A. R. BRAUN[3], A. MARTIN[2];
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Abstract: People with autism spectrum disorders (ASD) find face-to-face communication difficult. While previous neuroimaging studies have examined brain function in ASD during task and rest conditions and found abnormal differences in sensory, motor, social, and language networks, little is known about the function of these networks in an on-line, naturalistic conversation task. Here, we scanned 19 high-functioning autistics and 20 matched controls with fMRI while they conversed with an experimenter about their interests, hobbies, work and school life. Microphones, headsets and cameras were used to support face-to-face interaction. Using an unbiased, data-drive approach we found that the ASD participants showed greater whole-brain synchronization (timeseries co-variation) than the typically developed (TD) participants (voxelwise PTD) between all 24 regions revealed that most of the hyper-synchronization occurred within Network 2, and between Networks 2 and 3 ($P<.05$, two-tailed, Bonferroni corrected). Our results suggest that ASD brains may be less differentiated or functionally specialized than TD brains and that abnormal sensorimotor processing may relate to the difficulties ASDs have with face-to-face conversation.

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Poster

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Topic: C.06. Developmental Disorders

Support: NSF CMMI-1333841

Title: Developmental differences in functional connectivity in autism

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Abstract: Autism is a developmental disorder known to affect brain structure and function, but how it affects functional connectivity is not well understood. There is evidence for long-range hypoconnectivity and short range hyperconnectivity, but results are dependent upon control of motion, subject age, and methodological approach. In this study we synthesize two methodological approaches, graph-based functional connectivity (FC) and a novel method called network kernel analysis, to analyze within- and between-network connectivity of large-scale intrinsic functional networks. Our population consists of a cross-sectional sample (N=360) drawn from the Autism Brain Imaging Data Exchange (ABIDE) repository, selected based on root mean squared mean absolute motion < 0.5 mm, and individually matched for motion, full scale IQ, and age (7-35). We used literature-defined coordinates to identify nodes in the default mode network (DMN), dorsal attention network (DAN), frontal-parietal task control network (FPTC) and networks involved in emotional processing (SELF and OTHER)1. We censored motion-tainted frames. Using a graph approach, we found that within-network FC declined with age within the DAN, FPTC, and SELF networks. There was no main effect of diagnostic group on FC in any network after correction for multiple comparisons; however, the effect on OTHER approached significance (pcor = 0.056). There was no effect of age on between-network FC between SELF and OTHER and the remaining networks. Using network kernel analysis, and including nodes involved in salience, somotosensory and visual processing networks, we identified nine intrinsic network kernels whose structure was identical across five evenly distributed age bins and diagnostic group. A subsequent GLM using the network scores as regressors allowed us to map linear age-related developmental differences for each intrinsic network and examine group-related differences in slope. In general, network level developmental changes involved cortical specialization, lower cross-network correlation, and changes in cortical/subcortical connectivity. Developmental differences in ASD involve slower development of right-sided language-related homologues. We conclude that the network kernel approach allows us to consider group differences in connectivity in the context of substantial developmental network changes. References 1. Murray, R. J., Debbané, M., Fox, P. T., Bzdok, D. & Eickhoff, S. B. Functional connectivity mapping of regions associated with self- and other-processing. Human Brain Mapping 36, 1304-1324 (2015).

Disclosures: T.K. McAllister-Day: None. **T. Madhyastha:** None. **M. Reiter:** None. **M.K. Askren:** None. **N. Kleinhans:** None. **W.A. Chaovalitwongse:** None. **T. Grabowski:** None.

Poster

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Program#/Poster#: 680.11/F20

Topic: C.06. Developmental Disorders

Support: Wellcome Trust

Japan Society for Promotion of Science

Title: Association between age and rich-club structure in autistic and neurotypical human brains

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Abstract: Recent studies have shown the existence of rich-club organisation in large-scale anatomical human brain networks. The organisation consists of highly-connected regions and sparsely connected peripheral regions, and is supposed to contribute efficient information processing. In addition, disruption of this network organisation has been found in several psychiatric disorders including schizophrenia. In this study, using a publicly shared diffusion tensor imaging dataset, we found that brains of neurotypical individuals showed increases in rich-club architecture and network functionality during adolescence, whilst individuals with autism spectrum disorders did not. Moreover, this typical development of rich-club organisation was related with progressive involvement of the right anterior insula. In contrast, in autistic individuals, the anterior insula did not show typical increases in grey matter volume, and this anatomical immaturity was correlated with social symptoms of autism. These observations suggest that rich-club organisation is one of the bases of functionally efficient brain networks that enable complex cognitive functions in adult human brains, and imply that immature rich-club architecture might underlie some neurodevelopmental disorders.

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Poster

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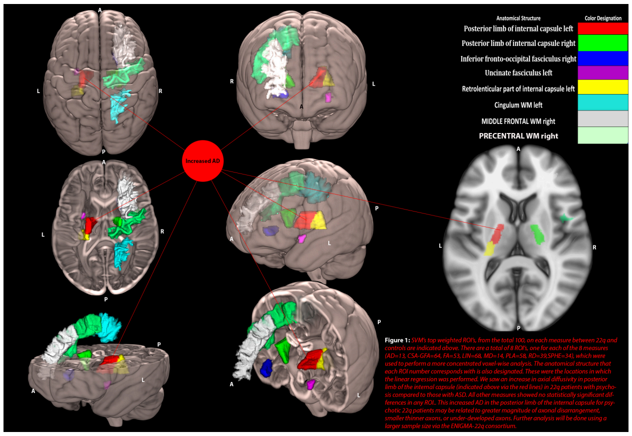
Topic: C.06. Developmental Disorders

Support: NIMH RO1 29032

Title: White matter microstructure differences in adolescents with autism, psychosis and 22q11.2 deletion syndrome

Authors: *J. GALVIS[1], J. E. VILLALON[1], G. PRASAD[1], C. CORBIN[3], T. M. NIR[1], L. KUSHAN-WELLS[4], C. E. BEARDEN[4], P. M. THOMPSON[2];
[1]USC Keck Sch. of Med., Marina Del Rey, CA; [2]USC Keck Sch. of Med., Los Angeles, CA; [3]USC, Los Angeles, CA; [4]UCLA, Los Angeles, CA

Abstract: 22q11.2 deletion syndrome (22q) is a neurogenetic disorder that can affect the heart, face, and brain. Many adolescents with 22q develop autism spectrum disorder (ASD), psychotic disorders (PS) or both. It is vital to understand which brain differences distinguish 22q from healthy developing adolescents and which pathological processes increase risk for ASD or PS. Here we analyzed white matter microstructural differences between 22q youth with (1) both ASD and Psychotic disorders, and (2) ASD only. We analyzed diffusion-weighted MRI (dMRI) scans using diffusion tensor imaging (DTI) and Q-ball imaging models to assess microstructural changes in the white matter. We computed DTI measures including axial, radial and mean diffusivity (AD, RD, MD), fractional anisotropy (FA), sphericity, linearity, planarity, and one orientation distribution function (ODF) based measure - the generalized fractional anisotropy - for all subjects (50 controls; mean age=12.48 years, M/F 29/21; and 46 22q patients; mean age=15.76 years, M/F 23/23). We computed the mean of each DWI derived measure in each of 100 white matter regions of interest, segmented using the Johns Hopkins white matter atlas. Support vector machines (SVMs) were used to classify individuals as 22q or healthy controls, and determine which ROIs had highest weight for classification. We obtained 92% accuracy with 10 fold cross validation. We performed a linear regression on each of the DWI measures within the ROIs ranked highest by the SVM algorithm to determine differences between 22q-ASD and PS. 22q youth with PS (n=12: mean age=16.25, M/F 5/7) had higher AD in the left posterior limb of the internal capsule, compared to ASD (n=12: mean age=12.91, M/F 8/4; FDR correction; $p < 7E-7$). For all other measures, we detected no significant differences between 22q individuals with and without psychosis. Higher AD in psychotic patients may be related to reduced axonal development. Via a new consortium, ENIGMA-22q, substantially increased sample sizes should identify more subtle but robust differences between these groups.



Disclosures: **J. Galvis:** None. **J.E. Villalon:** None. **G. Prasad:** None. **C. Corbin:** None. **T.M. Nir:** None. **L. Kushan-Wells:** None. **C.E. Bearden:** None. **P.M. Thompson:** None.

Poster

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Autism Science Foundation Grant 12-1001

Title: Relationship between language network connectivity and language deficits in children with Autism Spectrum Disorder

Authors: ***Y. GAO**[1], S. NAIR[2], A. LEGENKAYA[2], S. PUNYAMURTHULA[2], I. FISHMAN[2], R.-A. MÜLLER[2];
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Abstract: Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by sociocommunicative deficits. Studies suggest that atypical neural connectivity in ASD contributes to behavioral symptoms. However, evidence relating functional connectivity

of the language network to clinical measures of language in ASD remains limited. The present study examined links between intrinsic functional connectivity (iFC) of the language network and language abilities in children with ASD. Methods: We included 6-minute resting state fMRI scans from 31 ASD and 30 typically developing (TD) participants, ages 8-17 years. Groups were matched on age, non-verbal IQ, and head motion (all *p*s>.83). Data were preprocessed using nuisance regressors from six motion parameters, white matter and ventricles, and derivatives. Time points with motion >0.5mm and two subsequent time points were censored. All subjects included had >80% time points. Whole-brain iFC analyses were performed for seed in Broca's and Wernicke's areas in the left hemisphere (with coordinates adopted from Tomasi & Volkow, *Mol. Psychiatry* 2012). IFC maps were directly compared between the groups. Mean *z*-scores extracted from significant between-group clusters were correlated with scores from the Clinical Evaluation of Language Fundamentals (CELF). Results: Although within-group maps were mostly similar, direct group comparisons revealed significant iFC differences between ASD and TD groups (corrected $p<0.05$), with greater iFC (ASD > TD) between Broca's area and right middle temporal gyrus, and weaker iFC (ASD < TD) between Broca's area and right insula/frontal operculum. For Wernicke's area, extensive underconnectivity was detected in bilateral visual cortex. The latter was correlated with CELF Core Language scores ($r= -0.52$, $p<0.05$) and Expressive Language scores ($r= -0.51$, $p<0.05$) in the ASD group; i.e., greater iFC was associated with lower language scores. Conclusion: Patterns of atypical iFC in the ASD group differed between anterior and posterior perisylvian seeds. While overconnectivity between Broca's area and right middle temporal gyrus may relate to atypical functional asymmetry (increased right hemisphere participation) for language observed in some previous studies, extensive underconnectivity for Wernicke's area with striate and extrastriate visual cortices was a novel finding. Its potential significance was underscored by links with language ability. In particular, the link with expressive language was unexpected (given the primarily receptive role of Wernicke's area) and will require further investigation.

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Poster

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Topic: C.06. Developmental Disorders

Support: Lindamood-Bell Learning Processes

Title: Deficits in the brain's reading network in children with autism ameliorated by language remediation

Authors: *J. O. MAXIMO, D. L. MURDAUGH, A. R. LEMELMAN, C. E. CRIDER, S. E. O'KELLEY, R. K. KANA;
Psychology, Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Background: Individuals with autism spectrum disorders (ASD) may rely on intact or enhanced visuospatial skills to better facilitate language comprehension. Such compensation may underlie the engagement of neural circuitry that differs from typically developing individuals, or an underuse of the typical language-specialized regions in the brain. While attempts have been made to target language deficits in ASD using behavioral interventions, limited attention has been given to assessing intervention-related changes in brain functioning. **Objectives:** The objective of this study was to test the impact of an intensive visual imagery-based reading intervention on brain responses underlying language comprehension in children with ASD. **Methods:** The ASD participants were randomly assigned to a Wait-list control group (ASD-WLC; n = 10) and an Experimental group (ASD-EXP; n = 12). Both groups were scanned pre- and post-intervention, with only the ASD-EXP group receiving the intervention before their second scan. Participants went through a reading intervention program (Visualizing and Verbalizing for Language Comprehension and Thinking; 10-weeks, 200 hours of instruction). In the fMRI scanner, the participants performed 3 tasks: word comprehension, sentence comprehension, and verbal absurdity. **Results:** Across the 3 experiments: I) the ASD-EXP group showed an increase in bilateral motor, occipital and parietal activation compared to the ASD-WLC group post-intervention; II) intervention-related changes in brain activation were observed in the ASD-EXP group post-intervention in right cuneus, calcarine, and superior parietal lobule; and III) improvement (pre-to-post) in Gray Oral Reading Test scores significantly predicted changes in activation in the ASD-EXP group in precuneus, left angular, right IFG, and right insula. **Conclusions:** The findings of this study revealed that the reading intervention increased the brain activity in ASD-EXP children in regions associated with language and visual imagery. It should be noted that these results were accompanied by improvement in language comprehension in these participants. These findings underscore the potential of rigorous and structured interventions in targeting brain plasticity and improving functions in children with autism.

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Poster

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Topic: C.06. Developmental Disorders

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Autism Speaks

BioMarin Pharmaceutical, Inc.

Title: Global and modular resting state network topology in autism, phenylketonuria, and traumatic brain injury

Authors: *R. M. ZAMZOW, J. P. HEGARTY II, K. R. BELLESHEIM, M. H. PRICE, J. D. JOHNSON, G. YAO, D. Q. BEVERSDORF, S. E. CHRIST;
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Abstract: It has previously been suggested that functional connectivity, as measured by functional magnetic resonance imaging (fMRI), is altered in individuals with neurodevelopmental disorders or neurological syndromes. In the present study, we used graph theoretical analysis to examine how the topology of functional networks differs based on diagnosis. Resting state fMRI data was collected from 61 individuals with autism spectrum disorder (ASD) (mean age = 15.4), 12 individuals with phenylketonuria (PKU) (mean age = 23.6), 18 individuals with traumatic brain injury (TBI) (mean age = 39.4), and a comparison group of 61 typically developing individuals (TD) (mean age = 15.4). Partial correlation matrices for 90 cortical and subcortical regions were generated and thresholded across a range of network densities: $.05 \leq k \leq .48$. Topological properties were then compared between diagnostic groups and the TD group via area under the curve analyses. Statistical analysis revealed that the ASD group demonstrated reduced clustering coefficient ($p < .01$) as well as a trend toward increased characteristic path length ($p < .10$), as compared to the TD group. This pattern suggests disruptions in network efficiency at both the local and global levels in ASD. The PKU and TBI groups, however, displayed the opposite pattern. Both groups showed increased clustering coefficient ($p < .001$, in both instances) and reduced characteristic path length ($p < .001$, in both instances), as compared to controls, suggesting network-wide over-connectivity. In subsequent analyses, 13 functional subnetworks were identified for each group using a community detection algorithm and compared between groups for topological organization. In particular, as compared to the TD group, the ASD group demonstrated a reduced number of connections at each node within a subnetwork containing temporal cortical regions ($p < .01$). The PKU group showed reduced network efficiency and connection strength in a large subnetwork containing frontoparietal connections, as compared to the TD group ($p < .01$). Lastly, the TBI group displayed reduced network efficiency and connection strength in 9 of the 13 functional

subnetworks ($p < .01$ in all instances). The results of the present study indicate alterations in functional network and subnetwork topology that are distinct to specific diagnoses. Future studies are needed to characterize these alterations within the contexts of development and symptom severity.

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Poster

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Topic: C.06. Developmental Disorders

Support: MOST 103-2314-B-002 -030 -

Title: Motor imagery induced event-related desynchronization(ERD) of mu rhythm in ASD

Authors: ***Y.-T. CHEN**[1], K.-S. TSOU[2], C.-C. WONG[2], H.-L. CHEN[1], Y.-T. FAN[3], C.-T. WU[1,4];
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Abstract: Current evidence regarding whether the action representation is impaired in ASD remains inconclusive. While some studies showed that individuals with ASD demonstrate deficient action representation when performing action observation or imitation tasks, other studies showed the opposite results. In one of our recent behavioral study, we investigated the characteristic of action representation in ASD through motor imagery (MI) and showed that ASD is capable of performing kinesthetic MI but with a more inefficient way, reflecting preserved action representation in ASD. In the current study, we aimed to further investigate the neurophysiological mechanisms underlying MI in ASD through measuring MI-induced event-related desynchronization (ERD) in the mu rhythm (8-13 Hz). We recruited 16 individuals with ASD and 16 typically developing control (TDC) participants to perform a hand-rotation and an object-rotation task during EEG recording. In the hand-rotation task (involves kinesthetic MI), participants were required to judge the laterality of a 3-D model image of a bare-hand (the intransitive condition) or a hand-with-spoon (the transitive condition) that rotates with different

angles. In the object rotation task (involves object-based visual imagery), they were required to judge whether the drawer is on the right or on the left side of a desk that rotates with different angles. Specifically, to prevent contamination of mu power change induced by motor preparation and execution of manual responses, we modified our previous behavioral paradigm into a delayed response design. Based on the previous findings, we hypothesized that there would be positive biomechanical effects in which the medial rotation condition (comfortable position) would induce more mu ERD than the lateral rotation condition (awkward position), since the former is easier to imagine with kinesthetic information. For the hand rotation task, our results reveal that both groups demonstrated 'biomechanical effect' over the central scalp regions in the transitive condition. However, the mu power attenuations over the central regions of the ASD group were smaller than that of the TDC group both in the intransitive and transitive condition, for both medial (group difference for bare-hand: 8.6%; for hand-with-spoon:14.2%) and lateral rotation(group difference for bare-hand:12.4%; for hand-with-spoon, C3:11.6%). Our findings implicate that the inefficient performance of MI in individuals with ASD might result from reduced ERD in the mu rhythm over sensorimotor cortices.

Disclosures: Y. Chen: None. **K. Tsou:** None. **C. Wong:** None. **H. Chen:** None. **Y. Fan:** None. **C. Wu:** None.

Poster

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Autism Speaks Dennis Weatherstone Predoctoral Fellowship #7850

CDMRP AR093335

DOD10380424

Title: Links between thalamocortical and cerebrocerebellar intrinsic functional connectivity in autism spectrum disorder

Authors: *M. M. BERKEBILE[1], A. NAIR[2,1], A. J. KHAN[4], S. NAIR[1], M. DATKO[3], R.-A. MUELLER[1];

[1]Psychology, San Diego State Univ., San Diego, CA; [2]Clin. Psychology, [3]Cognitive Sci., UCSD, La Jolla, CA; [4]Clin. Psychology, Suffolk Univ., Boston, MA

Abstract: Introduction Research on autism spectrum disorder (ASD) has indicated atypical connectivity between cerebral cortex and deep structures such as thalamus and cerebellum. Recent intrinsic functional connectivity (iFC) work has shown predominant thalamocortical (TC) underconnectivity (Nair et al., Brain 2013), but cerebrocerebellar (CC) overconnectivity in ASD (Khan et al., Biol. Psychiatry 2015). However, these studies suggested similar domain-specific patterns of stronger iFC for sensorimotor (SM) and weaker iFC for supramodal (SU) connections. The present study examined these relationships by testing whether differential effects for SM and SU domains might be linked between TC and CC iFC. Methods 22 ASD and 27 typically developing (TD) participants, 7-17 years old, completed a 6 minute resting state scan (3T GE). Data were preprocessed in AFNI, with motion and fieldmap correction, spatial smoothing to 6mm3 FWHM, and Talairach normalization. Cortical regions of interest (ROIs) were obtained from the Jülich histological and Harvard-Oxford atlases. Partial correlation analyses were performed between mean time series from each unilateral cerebral cortical ROI and each ipsilateral thalamic and each contralateral cerebellar voxel. Results Our hypothesis linking domain-specific iFC patterns for TC and CC connectivity in ASD was not confirmed. One TC-CC correlation ($p<.05$) was found for left SM in the TD group (iFC between left SM cortex and left thalamus being positively correlated with iFC between left SM cortex and right cerebellum). The ASD group showed no such pattern. Examining individual ROIs, we found that this left SM correlation in the TD group was driven by perirolandic connectivity (M1, S1). In the ASD group, we found negative correlations ($r>.6$; $p<.005$) between CC iFC for right inferior/middle temporal cortex with TC iFC for right occipital and left S1 cortices; and a positive correlation between TC iFC for right posterior parietal and CC iFC for left superior temporal cortex. Conclusions Findings suggest that atypical TC and CC connectivity in ASD is not governed by a single principle of increased sensorimotor vs. reduced supramodal iFC. In fact, one link seen in the TD group for left SM was absent in the ASD group. Several atypical links were also seen in the ASD group. One of these showed that reduced cerebellar iFC with ventral visual stream (right inferior/middle temporal cortex) was linked to relatively increased thalamic iFC with right occipital cortex. This finding suggests that atypically increased activity and connectivity of early visual cortex observed in previous studies may be related to unusual subcortical and cerebellar connectivity.

Disclosures: **M.M. Berkebile:** None. **A. Nair:** None. **A.J. Khan:** None. **S. Nair:** None. **M. Datko:** None. **R. Mueller:** None.

Poster

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Psychology Department Senior Thesis Fund (Department of Psychology at Princeton University)

Title: Attention-based learning deficits in individuals with autism suggest constitutively elevated norepinephrine levels

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Abstract: Autism spectrum disorders comprise a class of conditions characterized in part by a deficit in sensory integration (Lai, Lombardo, & Baron-Cohen, 2013). Previous work suggests that the degree to which learning about environmental stimuli integrates over different stimulus features is modulated by neural gain. High gain corresponds to tonic activity of the locus coeruleus (LC) and increased global levels of norepinephrine (NE) levels in the brain, and has been associated with a less integrative mode of learning and processing. In contrast, low gain correlates with LC phasic activity, resulting in decreased NE concentrations. That is, when neurotypical individuals are in a high gain state, they have a tendency to narrow their attentional breadth and learn from few stimulus dimensions that they are particularly predisposed to, whereas in a state of low gain they are able to learn from multiple stimulus features simultaneously (Eldar, Cohen, & Niv, 2013). Given autistic individuals' deficits in attention-based learning and relatively large baseline pupil diameters (an indicator of enhanced NE release from LC terminals), we tested the behavioral performance and pupillary responses of autistic and neurotypical teenagers on a task measuring the extent of learning from multidimensional stimuli. While autistic participants' pupillary responses were consistent with a high gain state, the behavioral data suggested that autistic individuals were more likely to learn from multiple stimulus dimensions, as compared to neurotypical controls. To reconcile these opposing findings, we propose that in the autistic brain, chronically elevated levels of NE cause desensitization of adrenergic receptors, thereby resulting both in a significant diminishment of gain and a decrease in NE's capability of modulating the signal-to-noise ratio of a range of neural circuits. In contrast, adrenergic receptors controlling pupil dilation are less susceptible to desensitization

(Heck & Bylund, 1998), and thus reveal the elevated NE levels more directly. Our results thereby support a possible neural basis for deficits in attention-based learning in autistic individuals, while also accounting for several classic autistic features. References Eldar, E., Cohen, J. D., & Niv, Y. (2013). The effects of neural gain on attention and learning. *Nature Neuroscience*, *16*, 1146-1153. doi: 10.1038/nn.3428 Heck, D. A., & Bylund, D. B. (1998). Differential down-regulation of alpha-2 adrenergic subtypes. *Life Sciences*, *62*, 1467-1472. doi: 10.1016/S0024-3205(98)00091-5 Lai, M.-C., Lombardo, M. V., & Baron-Cohen, S. (2013). Autism. *Lancet*, *383*, 896-910. doi: 10.1016/S0140-6736(13)61539-1

Disclosures: M.C. Granovetter: None. **E. Eldar:** None. **Y. Niv:** None.

Poster

680. Autism: Physiology and Systems

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Topic: C.06. Developmental Disorders

Support: NIH/NIMH Intramural Research Program

Brain & Behavior Research Foundation, NARSAD Young Investigator Grant

Title: Resting-state functional connectivity predicts prospective change in social functioning and adaptive behaviors

Authors: *M. PLITT[1], K. A. BARNES[2], G. L. WALLACE[2], L. KENWORTHY[2], A. MARTIN[2];
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Abstract: Though typically identified in early childhood, the social communication symptoms and adaptive behavior deficits that are characteristic of autism spectrum disorder (ASD) persist throughout the lifespan. Despite this persistence, even high-functioning individuals show substantial heterogeneity in outcomes. Previous studies have found various behavioral assessments such as IQ, early language ability, and baseline social functioning and adaptive behavior scores to be predictive of outcome, but most of the variance in functioning remains unexplained by such variables. In this study, we investigated to what extent functional brain connectivity measures obtained from resting-state functional connectivity MRI (rs-fcMRI) could predict the variance left unexplained by behavior (age, follow-up latency, and baseline social

functioning and adaptive behavior scores) in two measures of outcome—adaptive behaviors and social functioning at least one year post-scan (mean follow-up latency = 2 years 10 months). We found that connectivity involving the so-called salience network (SN), default-mode network (DMN), and frontoparietal task control network (FPTCN) was highly predictive of future social functioning and the change in social functioning and adaptive behavior over the same time period. Furthermore, functional connectivity involving the SN, which is predominantly composed of the anterior insula and the dorsal anterior cingulate, predicted reliable improvement in adaptive behaviors with 100% sensitivity and 70.59% precision (the ability of a model to not label a scan as positive when the true label is negative). Our study successfully predicted heterogeneity in outcomes for individuals with ASD from rs-fcMRI data that was unaccounted for by simple behavioral metrics and provides novel evidence for networks underlying long-term symptom abatement.

Disclosures: **M. Plitt:** None. **K.A. Barnes:** None. **G.L. Wallace:** None. **L. Kenworthy:** None. **A. Martin:** None.

Poster

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Support: Max Kade Foundation

ISNR Research Foundation

Title: Investigating the effects of Neuro Feedback training on the functional brain connectivity of children on the autism spectrum

Authors: ***A. COURELLI**[1], H. COURELLIS[1], E. FRIEDRICH[2], J. A. PINEDA[2];
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Abstract: Neuro feedback training (NFT) constitutes a promising treatment for ASD that has been shown to produce positive behavioral changes. The majority of the analyses regarding the effects of NFT on the behavior of autistic children have relied on caretaker surveys and changes in the power of EEG frequency bands. While such analyses provide benchmarks regarding NFT effectiveness, they do not provide information about changes in functional brain connectivity that can be used to explain behavioral changes. To investigate changes in functional brain

connectivity, we computed Granger Causality spectral estimates using filtered EEG signals recorded from pediatric ASD patients before and after NFT. Closed loop NFT was conducted on 13 autistic children, ages 8-17, with the aim to upregulate mu-rhythms, 8-13 Hz rhythms in the sensorimotor cortex, and suppress beta and theta rhythms. Prior to the beginning of the NFT and after 16 sessions were completed, an emotion recognition test was conducted and EEG data were recorded using a 32 electrode full cap EEG. Patients were shown a series of video clips of individuals making happy, fearful, or angry faces. The videos were categorized into two stimulus classes according to the type of emotion they presented: positive (happy) and negative (fearful/angry).The acquired data were processed by applying artifact rejection, the cleaned data were epoched to encompass only the video presentation stimuli, and Independent Component Analysis (ICA) was conducted to identify sources of electrical activity during the epoch. Non-artifactual Independent Components (IC's) selected for the positive and negative stimulus class served as nodes for the functional brain connectivity networks associated with each stimulus class. Using the selected ICs, a Multivariate Autoregressive model was constructed for each stimulus class and spectrotemporal Granger causality (GC) was computed among all possible pairs of the selected IC's. Changes in the strength of the causal connections were subsequently determined by averaging spectral GC power within the trained frequency bands across the entire epoch. Key brain regions associated with ICs that exhibited change in causal connection strength for each stimulus class corresponded to centers for self-regulation and social cognition, visual processing centers, and the pre motor cortex. Caretaker evaluations of the patients' performance pre and post NFT (Social Responsiveness Scale and Autism Treatment Evaluation Checklist) suggested that the observed changes in functional brain connectivity were accompanied by improvement in social responsiveness and a decrease in autistic symptoms.

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Poster

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Topic: C.06. Developmental Disorders

Support: NIH/NIMH MH096582

Title: Are sensory problems in autism really sensory?

Authors: M. ZINNI[1,4], M. WESTERFIELD[2], S. WEE[3], L. CHUKOSKIE[2], *J. TOWNSEND[1]; [1]Dept Of Neurosci, [2]Inst. Neural Computation, [3]Dept. Neurosciences, UCSD, La Jolla, CA; [4]Neuroverse, Inc., San Diego, CA

Abstract: Abnormal responses to sensory stimulation are a commonly reported clinical feature of autism spectrum disorder (ASD). Both over- and under-responsiveness have been reported in all sensory modalities, although the nature and affected sensory modality of these abnormalities varies greatly between individuals. Despite the general consensus that sensory abnormalities are an important clinical symptom in autism, there is little or no understanding of the underlying mechanisms. Because the vast majority of evidence regarding these sensory symptoms in ASD are based solely on behavioral descriptions, it is not clear whether these symptoms are related to differences in function at a basic sensory level or whether these symptoms result instead from the influence of poorly modulated arousal and attention on sensory responsiveness. The present study examined these factors in adults with ASD compared to typically developing (TD) adults. Participants completed three experiments utilizing behavioral, event-related brain potential (ERP) and heart-rate (HR & HRV) measures to collectively examine electroencephalographic (EEG) sensory responses to: auditory stimulation; changes in intensity of auditory stimulation; repeated auditory stimulation (refractory and habituation responses). During these experiments we also examined the effects of physiological arousal and attention on brain responses to sensory stimulation. We found significant sensory problems on the Sensory Profile Questionnaire in ASD adults, suggesting that sensory difficulties persist throughout the lifespan. While ASD participants reported more sensory difficulties, our physiological measures did not support a true sensory origin of these difficulties. The ERP responses to auditory stimuli of varying intensity were similar in amplitude between ASD and TD groups. Also, ASD adults, like TD adults showed the expected refractory response to a repeated sensory stimulus (i.e., the response to the second of a pair of stimuli with differing inter-stimulus intervals was smaller). However, gradual habituation over time was atypical in ASD, but only when sensory stimulation was unattended. Additionally, baseline arousal differed between groups as measured by HR & HRV as well as greater arousal in the ASD group during the presentation of auditory tones. These findings suggest that in adults with ASD, sensory difficulties that are experienced in daily life may be a function of differences in the modulation of general arousal and the effects of attentional state rather than abnormalities in basic sensory response.

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Poster

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Topic: C.06. Developmental Disorders

Support: University of Michigan MCubed Initiative

Title: Relationship between neural coherence and attention in autism spectrum disorder

Authors: A.-M. FLORES[1], T. ANDERSEN[1], C. SWICK[1], R. GOODCASE[1], J. BRENNAN[2], I. KOVELMAN[2], S. BOWYER[3], *R. LAJINESS-O'NEILL[1];
[1]Eastern Michigan Univ., Ypsilanti, MI; [2]Univ. of Michigan, Ann Arbor, MI; [3]Henry Ford Hlth. Systems, Detroit, MI

Abstract: Autism Spectrum Disorder (ASD) is characterized by atypical attentional functioning. Aberrant neural synchrony may be a neurophysiological mechanism underlying this characteristic. This study investigated relationships between synchrony and attention. Twelve ASD (Age: M = 8.9; SD= 1.0) and 13 neurotypical (NT) children (Age: M = 9.3; SD = 1.3) underwent magnetoencephalography (MEG) at rest. Synchronization was quantified by calculating coherence between cortical sites. Kendall Tau correlations were computed to examine relationships between coherence and auditory attention (AA). Alpha-band: In ASD, coherence between cingulate and orbitofrontal regions was positively related to shifting errors (τ=-0.73- -0.60). In NT, higher coherence between frontal and subcortical regions, and from the cingulate to fronto-parietal regions, was related to better AA (τ=0.48- 0.74). Gamma-band: In ASD, coherence between fronto-temporal regions, and temporal lobes, was positively related to AA (τ=0.47- 0.51). Coherence between temporal and pre/postcentral regions was negatively related to AA inhibition (τ=-0.47). Increased connectivity was related to better AA, shown by fewer omission errors (τ=0.45-0.65). Connectivity from orbitofrontal to all other regions was related to higher impulsivity on set shifting tasks (τ=-0.66- -0.47). In NT, increased coherence between fronto-parietal regions was related to less AA inhibition (τ=-0.63- -0.48); coherence between orbitofrontal and temporal regions was positively related to inhibition (τ=0.48- 0.56). Higher coherence between fronto-temporal regions was related to more omission errors on a set shifting task (τ=-0.61- -0.50). Coherence between bilateral temporo-parieto-occipital regions was negatively related to AA impulsivity (τ=-0.60- -0.48). Enhanced alpha connectivity between cingulate and orbitofrontal regions suggested poor regulation in ASD. Enhanced alpha connectivity in NT between frontal and subcortical regions and from cingulate to fronto-parietal regions, enhanced AA. Better AA performance in ASD were related to increased gamma connectivity between temporal and fronto-temporal regions. Poor inhibition was differentially associated in ASD and NT with increased gamma connectivity between temporal and pre/postcentral regions and frontoparietal regions, respectively. Better inhibition was associated with increased gamma connectivity between orbitofrontal and temporal regions in NT. Overall,

posterior connectivity was associated with impulsivity. Findings suggest differential cortical connectivity in ASD and NT associated with auditory attention.

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Poster

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Support: Autism Speaks Grant 2718

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DoD Grant W81XWH-10-1-0474

Title: Brainstem morphometry in individuals with and without autism spectrum disorders

Authors: *S. E. CHRIST, M. C. ABRAHAM, G. YAO, D. Q. BEVERSDORF;
Univ. of Missouri, Columbia, MO

Abstract: Objective: Previous studies of brainstem morphometry in individuals with autism spectrum disorder (ASD) have yielded mixed results. Whereas some studies (e.g., Gaffney et al. 1988, Jou et al. 2013) have found ASD-related abnormalities, others (e.g., Elia et al, 2000) have reported no significant differences between individuals with and without ASD. The objective of the present study was to further examine this issue while also investigating the relationship between morphometry and autistic symptomatology. To this end, structural volume of the midbrain, pons, and medulla were studied in a sample of adolescents and young adults with and without ASD. Methods: High resolution T1-weighted MRI structural images were acquired from 20 individuals with ASD (male/female: 18/2; mean age: 13.0 yrs) and an age- and gender-matched comparison group of 21 typically developing individuals without ASD (male/female: 20/1; mean age: 13.1 yrs). All data processing was conducted by individuals who were masked to the group status of the participants. Following acquisition, the structural MR image data for each participant was rotated into AC-PC coordinates, and the brainstem structures of interest (i.e., midbrain, pons, and medulla) were then manually segmented in a standardized fashion to obtain precise volumetric measurements. Manual segmentation, while time-consuming, is considered the “gold-standard” by most investigators for detecting subtle volumetric differences

(Harms et al., 2010). Results & Conclusions: Overall, there was a trend towards larger total brain stem volume in the ASD group (M = 29.43 cm^3) compared to the typically developing non-ASD group (M = 28.05 cm^3); however, this difference did not rise to statistical significance, $t(39)$ = 1.57, p = .13, d = .50. Similar non-significant trends were observed for all three individual structures of the brainstem (midbrain, pons, and medulla). Of note, within the ASD group, a relationship (r = .54, p = .02) between brainstem volume and autistic symptomatology (as measured by the Social Communication Questionnaire; Rutter et al., 2003) was evident. This relationship held for all three individual structures (midbrain: r = .52; pons: r = .44; medulla: r = .53). The present findings replicate and extend previous studies which have reported ASD-related abnormalities in brainstem morphometry (Gaffney et al. 1988, Jou et al. 2013). Additional research is needed to examine the extent to which the presently the observed relationship between brainstem volume and autistic symptoms may be driven by a particular aspect of symptomatology (e.g., sensory abnormalities).

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Poster

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Topic: C.06. Developmental Disorders

Title: Gamma band oscillations to reveal neural network cortical coherence dysfunction in autism spectrum disorder

Authors: *K. HUANG[1,2], Y.-T. LIN[3], S. S. F. GAU[2,4];
[1]Natl. Taiwan Univ. Hosp., Taipei, Taiwan; [2]Grad. Inst. of Brain and Mind Sciences, Natl. Taiwan Univ., Taipei, Taiwan; [3]Dept. of Psychiatry, Natl. Taiwan Univ. Hospital, Taipei, Taiwan, Taipei, Taiwan; [4]Dept. of Psychiatry, Natl. Taiwan Univ. Hospital, Taipei, Taiwan, Taipei, Taiwan, Taipei, Taiwan

Abstract: Objective: Autism spectrum disorder (ASD) is a neurodevelopment disorder involving γ-aminobutyric acid (GABA). Sensory abnormalities, particularly in the auditory modality, are commonly seen in individuals with ASD. Since GABA system plays a key role in generating neuronal gamma oscillations, this study aims to investigate whether ASD patients have impairments in gamma-band auditory steady-state response (ASSR) which is an auditory event-related potential. **Method**: Twenty-nine children and adolescents with ASD, and twenty-

eight typical developing (TD) children and adolescents were recruited. Subjects were presented three click trains with rates of stimulation at 20, 30 and 40 Hz, respectively. The ASSR was recorded through a 32-channel electrode cap. Single-electrode ERSP (Event-Related Spectral Perturbation) and ITC (Inter-Trial phase Coherence) indices were derived from the electroencephalogram (EEG) signals. **Results**: The ASD group showed higher ERSP power in the frontal and parieto-occipital region and smaller ITC in right temporal area, in response to 20 Hz click trains. Regarding 30 Hz click train stimulation, lower ERSP at electrode CP3 and smaller ITC at F4, T4, were observed in ASD group. The mean of ERSP to 40 Hz click train was higher at C4 and P4 in ASD group. **Conclusion**: These findings suggested different auditory responses to train stimulation in subjects with ASD. However, group differences in the gamma-band EEG responses were not that obvious. Larger sample is needed to better explore the ASSR in ASD subjects.

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Poster

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Title: An altered divisive normalization model of autism

Authors: *J. PATTERSON, A. ROSENBERG, D. ANGELAKI;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Autism is a neurodevelopmental disorder marked by a diverse set of symptoms including perceptual, social, and cognitive atypicalities. This heterogeneity presents a significant challenge to establishing a comprehensive characterization of the disorder. The widespread effect of the disorder on neural systems suggests that autism may broadly impact neural computations as opposed to isolated systems. As such, we hypothesize that alterations in canonical computations that occur throughout the brain may underlie the behavioral characteristics of autism. Here we focus on one computation in particular, divisive normalization, which balances a neuron's net excitation with inhibition reflecting the combined activity of a population of

neurons. Divisive normalization inherently reflects the ratio of neural excitation to inhibition, which is believed to be abnormally elevated in autism. In the present work, we show that an altered divisive normalization signal which elevates the excitatory/inhibitory ratio can account for perceptual findings in autism. Specifically, we develop a neural network model of primary visual cortex (V1) in which individual units are selective for stimulus location and orientation. An increased E/I ratio is simulated in the model by reducing the strength of the inhibitory divisive normalization signal reflecting the population activity. To examine how this alteration might give rise to perceptual autism symptomatology, we simulate two perceptual studies comparing the behavior of typically developing controls and individuals with autism on tasks that strongly engage V1. The first, a motion discrimination task employing stimuli of different sizes and contrasts, revealed reduced surround suppression and overall better discrimination performance in autism for high contrast stimuli, but equivalent performance across the groups at low contrasts. The second, a feature detection task investigating the facilitating effect of an attentional cue, reported a sharper gradient of attention in autism than in controls. Interestingly, we find that the results of both studies could be accounted for by the same alteration in divisive normalization. Our results suggest that the divisive normalization framework can provide novel insights into the neural basis autism and generate hypotheses that are readily testable by psychophysics experiments. In future work, it should be possible to adapt this framework to other sensory modalities as well as more complex operations such as facial processing which require hierarchical processing.

Disclosures: **J. Patterson:** None. **A. Rosenberg:** None. **D. Angelaki:** None.

Poster

681. Down Syndrome

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Jerome Lejeune Foundation

Title: Environmental and pharmacological intervention restores cognitive impairment in a mouse model of Down syndrome

Authors: *M. DIERSSEN, S. CATUARA;
CRG - Ctr. For Genomic Regulation, Barcelona, Spain

Abstract: Down syndrome (DS) is caused by trisomy of chromosome 21 (HSA21) leading to cognitive disability and high prevalence of Alzheimer-type dementia. Currently there is no effective therapy to improve learning and memory or to prevent/delay neurodegeneration later in life. Studies in a trisomic mouse model of DS (Ts65Dn) have indicated that environmental enrichment (EE), a housing condition that enhances social, sensorimotor and cognitive abilities, promotes neuroplasticity effects that can partially rescue cognitive impairments but the effects are limited and temporary. The dual specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A) has been postulated as a primary candidate gene located in chromosome 21 and implicated in multiple DS phenotypes. It encodes a serine-threonine kinase that phosphorylates critical substrates in neuroplasticity, cognitive function and neurodegeneration. We previously showed that Dyrk1A kinase levels and activity are normalized to wild type levels by EE in DS mouse models. Here we tested if the inhibition of Dyrk1A activity using (−)-Epigallocatechin-3-Gallate (EGCG), a potent Dyrk1A inhibitor, potentiates the effects of EE inTs65Dn mice in young animals and at older ages associated with the onset of neurodegeneration by analyzing the effect of a combined EGCG-EE intervention on the cognitive performance of Ts65Dn mice. Our results indicate that the combined use of EGCG-EE significantly improves visuo-spatial and recent memory, a cholinergic-dependent cognitive function in young and middle-age trisomic mice. The data indicate that combined use of EGCG-EE in trisomic mice potentiates the effects of EE, inducing different beneficial effects during lifetime.

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Poster

681. Down Syndrome

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IUPUI Dept. of Psychology Internal Support Grant

Title: Deficits in a radial-arm maze spatial pattern separation task in a mouse model for Down syndrome

Authors: M. E. STRINGER[1], I. ABEYSEKERA[2], R. J. ROPER[2], *C. R. GOODLETT[3]; [1]Psychology, [2]Biol., [3]IUPUI, Indianapolis, IN

Abstract: Down syndrome (DS) is caused by three copies of human chromosome 21 (Hsa21) and results in a constellation of phenotypes including intellectual disability. Ts65Dn mice, the most extensively studied DS model, have three copies of ~50% of the genes on Hsa21 and display many phenotypes associated with DS, including cognitive deficits. DYRK1A is found in three copies in humans with Trisomy 21 and in Ts65Dn mice, and is involved in a number of critical pathways including CNS development and osteoclastogenesis. Epigallocatechin-3-gallate (EGCG), the main polyphenol in green tea, inhibits Dyrk1a activity. We have shown that a three-week EGCG treatment (~10mg/kg/day) during adolescence normalizes skeletal abnormalities in Ts65Dn mice, yet the same dose did not rescue deficits in the Morris water maze (MWM) or novel object recognition (NOR). In contrast, a higher EGCG dose (90mg/kg/day) was reported to improve performance on MWM and NOR in Ts65Dn mice. The current study investigated a spatial memory pattern separation task in Ts65Dn mice, a hippocampal-dependent task that requires differentiation between similar memories acquired during different learning episodes. Distinguishing between these similar memories is thought to depend on distinctive encoding in the hippocampus linked to functional activity of newly generated granule cells in the dentate gyrus. Given that recent studies in Ts65Dn mice have reported significant reductions in adult hippocampal neurogenesis, we hypothesized that Ts65Dn mice would be impaired in the pattern separation task, and that EGCG would alleviate the pattern separation deficits seen in trisomic mice in association with increased adult hippocampal neurogenesis. Mice were given EGCG (~100 mg/kg/day) or water beginning on postnatal day (PD) 24 and trained beginning on PD 75 on a radial-arm maze delayed non-matching-to-place pattern separation task that included three different degrees of spatial separation. Euploid mice performed significantly better over training than Ts65Dn mice, including better performance at each of the three separations. EGCG did not appear to alleviate the deficits in Ts65Dn mice. Assessment of adult neurogenesis in these mice is ongoing. This is the first study to show deficits in Ts65Dn mice on a pattern separation task. To the extent that pattern separation depends on the functional involvement of newly generated neurons in an adult dentate gyrus, this approach in Ts65Dn mice may help identify more targeted pharmacotherapies for cognitive deficits in individuals with DS. Supported by an IUPUI Research Support Funds Grant (to RJR and CRG) and Department of Psychology Internal Support Funds (CRG) from IUPUI.

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Poster

681. Down Syndrome

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Ohio Department of Developmental Disabilities

Awakening Angels Foundation

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/MCTI)

Title: Delayed maturation of visual system in the mouse model of Down syndrome Ts65Dn

Authors: M. R. STASKO, D. B. VICTORINO, J. J. SCOTT-MCKEAN, B. L. ZAMPIERI, *A. C. COSTA;
Div. of Pediatric Neurology, Dept. of Pediatrics, Case Western Reserve Univ., Cleveland, OH

Abstract: Down syndrome (DS), which is caused by the trisomy of chromosome 21, is the most common genetically defined cause of intellectual disability. This genetic disorder also affects the visual system in a variety of ways, which include high incidence of refractive errors, accommodative inaccuracy, amblyopia, strabismus, nystagmus, abnormal oculomotor and vestibular functions, decreased visual acuity, and decreased color and contrast sensitivities. In a previous study (Scott-McKean et al., IOVS 51: 3300- 3308, 2010), we demonstrated that adult mice Ts65Dn (a murine model of DS) exhibit deficits in luminance threshold, spatial resolution, and contrast threshold, compared with euploid control mice, as assessed electrophysiologically by pattern visual evoked potentials. Here, we investigated visual thresholds of optokinetic tracking (OKT), a fundamental visual behavior that facilitates the relative stabilization of retinal images. Using the methods originally described in detail by Prusky el al. (IOVS 45:4611-4616, 2004), we were able to quantify OKT thresholds in untrained and freely moving Ts65Dn and control euploid mice, daily from eye opening (postnatal day 14) to 35 days of age, and then in longer intervals (5-10 days), until the animals were 60-day old. We found that Ts65Dn mice show a significant delay in the maturation of the visual system. Whereas the mean spatial frequency sensitivity to a 100% contrast grating projected on a virtual cylinder for 14-day old euploid control mice was 0.21 c/deg, the mean value of this measure was 0.08 c/deg for Ts65Dn mice. Ts65Dn mice were only able to achieve a mean spatial frequency sensitivity of 0.21 c/deg at 16 days of age. At age 45 days, the measured values of mean spatial frequency sensitivity were 0.45 and 0.43 c/deg for control euploid and Ts65Dn mice, respectively. The development of contrast sensitivity was also delayed in Ts65Dn mice. At 17 and 35 days of age, contrast sensitivity was 6% and 17% in Ts65Dn mice versus 9% and 26% in control mice, respectively. We are currently comparing these behavioral based findings with *in vivo* electrophysiological assessments by means of patterned visually evoked potentials. In addition, the integrity of the

visual path is being assessed through the investigation of potential lenticular and/or retinal lesions with a slit lamp imaging system and a retinal imaging microscope equipped with optical coherence tomography capabilities. The observed delay in the maturation of the visual system in a mouse model of DS mimics the qualitative features of the same phenomenon seen in young persons with DS.

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Poster

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Support: NIH, NICHD/NIMH, RO1HD05780

Title: Spinal cord abnormalities in the Ts65Dn mouse model of Down syndrome

Authors: ***N. M. AZIZ**, J. L. OLMOS-SERRANO, T. F. HAYDAR;
Boston Univ. Sch. of Med., Boston, MA

Abstract: Individuals with Down syndrome (DS) are severely hypotonic at birth and exhibit disturbances in movement production and postural control during early postnatal development. As such, acquisition of motor skills is significantly delayed in DS patients, negatively impacting their gait and fine motor control throughout life. Work in our lab has for the first time elucidated substantial cytoarchitectural as well as gene expression abnormalities in spinal cords (SCs) of Ts65Dn mice. Because the full spectrum of SC deficits in DS is still unknown, the impact of trisomy on motor neurons (MNs), interneurons (INs), and oligodendrocytes (OLs) was analyzed both pre- and postnatally. Our qRT-PCR data show a 144.7±60% increase in expression of the homeodomain transcription factor *Hb9*, which is found in MNs and in a subset of INs, in postnatal day 60 (P60) Ts65Dn mice compared to euploids. This increase was accompanied by a 47.0±13% decrease in the expression of the IN-specific homeodomain transcription factor *Irx3*. Immunohistochemical (IHC) assessment of OL maturation in a spinal white matter tract showed enrichment in immature versus mature OLs at both P30 and P60. Upon further investigation of the white matter, we also found a decrease in the number of nodes of Ranvier at P30 in Ts65Dn mice compared to euploids. Concomitant with these gene expression and histological differences were profound deficits in hanging wire and hindlimb reflex performance in the Ts65Dn mice.

Along with the reported postnatal aberrations, prenatal abnormalities in gene expression were also detected at embryonic day 12.5 (E12.5) and E14.5 in SCs of Ts65Dn mice. This includes a 95.7±26% increase in expression of *oligodendrocyte transcription factor 2* (*Olig2*) and a 24.3±6% increase in *Hb9* expression at E12.5. Olig2+ cell numbers were also significantly increased in T65Dn SCs at this age. At E14.5, expression of *Hb9* and *Olig2* was normalized, yet expression of another patterning transcription factor known as *Nkx2.2* was decreased by 27.1±7% in SCs of trisomic animals. Collectively, our data show that population dynamics and gene expression in spinal MNs, INs and OLs are affected in both postnatal and embryonic Ts65Dn, suggesting that widespread and temporally unrestricted aberrations exist in trisomic SCs. Importantly, data also show that OL maturation and function are adversely impacted, pointing to a novel link between Down syndrome and improper myelination in the SC. These observed defects are accompanied by deficits in reflexive motor tasks such as the hanging wire and hindlimb reflex in Ts65Dn mice.

Disclosures: **N.M. Aziz:** None. **J.L. Olmos-Serrano:** None. **T.F. Haydar:** None.

Poster

681. Down Syndrome

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 681.05/F39

Topic: C.06. Developmental Disorders

Title: Endogenous DNA damage and cellular death pathways in Down Syndrome

Authors: *A. PINTO[1], M. M. SERAFINI[1], C. LANNI[1], S. GOVONI[1], M. RACCHI[1], E. PROSPERI[2], D. NECCHI[1];
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Abstract: Background: Down syndrome (DS) is a genetic disease due to triplication of genes located on chromosome 21. DS phenotype is complex and includes mental retardation, increased incidence of congenital heart disease, hypothyroidism, leukemia and pulmonary hypertension. No single gene or region of human chromosome 21 is responsible for all common features of DS. More likely, interactions between various genes, their altered expression and other factors could better explain the major DS phenotypes and their severity. Moreover, Down syndrome is characterized by genetic instability, neurodegeneration and premature aging. However, the molecular mechanisms leading to this phenotype are not yet well understood. We observed that DS fibroblasts from both fetal and adult donors show the activation of a DNA damage response already during unperturbed growth conditions, as indicated by histone H2AX and

phosphorylation of checkpoint protein. Furthermore, increased levels of p53 protein in untreated DS fibroblasts were observed, although DNA damage was not investigated. Interestingly, we also found an altered conformational state of p53 in Ts65Dn brain, thus resulting in an impaired and dysfunctional response to stressors. **Objective:** To examine the transcriptional function of p53 in the context of neurological disorder and then the consequences of unfolded p53 on its activity in Down Syndrome. Our long-term goal is to elucidate the role of unfolded p53 in human brain disease progression. **Methodology:** Fetal and adult fibroblasts were used to understand whether DNA damage response activation was related to damage accumulation with age or to genetically inherited properties. To study alterations of cell death pathways induced by conformationally altered p53, we used Ts65Dn mouse model, the most investigated DS model with a triplicated segment of orthologous human chromosome 21. Considering the current notion that autophagy plays a critical role in multiple pathological lesions we focused our attention on p53-related autophagy pathways to better understand their involvement in the development of neurodegeneration in DS individuals. **Conclusions:** We have recently investigated the presence of endogenous DNA damage and a defective repair mechanism in fetal and adult DS fibroblasts that could contribute to genome instability in Down Syndrome. Furthermore, animal DS model shows a conformational altered status of p53, a key mediator of the DNA damage response. As a consequence, p53 is not able to induce the expression of a group of target genes involved in several pathways to maintain genome stability in response to stress signals, thus contributing to the system dysfunction.

Disclosures: **A. Pinto:** None. **M.M. Serafini:** None. **C. Lanni:** None. **S. Govoni:** None. **M. Racchi:** None. **E. Prosperi:** None. **D. Necchi:** None.

Poster

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Topic: C.06. Developmental Disorders

Support: AG043375

AG014449

AG017617

Alzheimer's Association IIRG-12-237253

Title: Microarray analysis of entorhinal cortex stellate cells in the Ts65Dn mouse model of Down syndrome and Alzheimer's disease following maternal choline supplementation (MCS)

Authors: *H. M. CHAO[1], M. J. ALLDRED[2,5], S. LEE[3], E. PETKOVA[4,6], S. D. GINSBERG[2,5,7];
[2]Ctr. for Dementia Res., [3]Med. Physics, [4]Child Psychiatry, [1]Nathan Kline Inst., Orangeburg, NY; [5]Psychiatry, [6]Child & Adolescent Psychiatry, [7]Neurosci. & Physiol., New York Univ. Langone Med. Ctr., New York, NY

Abstract: Down syndrome, caused by trisomy of chromosome 21, is characterized by physical and cognitive impairments, and by early presentation of Alzheimer's disease (AD) pathology. The trisomic Ts65Dn mouse model of Down syndrome (DS) and AD exhibits many key phenotypes of the disorder including learning and memory deficits and basal forebrain cholinergic neuron (BFCN) degeneration. Choline is an essential nutrient required for proper central nervous system development of the fetus, and maternal choline supplementation (MCS) has been shown to be beneficial to both normal disomic (2N) and trisomic (Ts65Dn) offspring with regard to adult hippocampal neurogenesis and performance on attentional and cognitive tasks. The entorhinal cortex, as a gateway to and from the hippocampus, has an essential role in executive function and memory, and has been shown to be particularly vulnerable in aging, DS, and Alzheimer's disease (AD).In the present study we utilize custom-designed microarrays with subsequent Nanostring nCounter and qPCR validation to investigate entorhinal cortex gene expression from layer II/III stellate cells in Ts65Dn and 2N littermates. Specifically, offspring from both choline supplemented dams (~4 times the normal dietary intake of choline) and normal choline fed dams were analyzed at 5-7 months of age.. Briefly, individual stellate cells were microaspirated via laser capture microdissection (LCM) and then subjected to terminal continuation (TC) RNA amplification and analysis on a custom-designed microarray platform with ~576 genes relevant to neurodegeneration and neuroscience. Validation via Nanostring nCounter and qPCR is performed on regional dissections of the entorhinal cortex from Ts65Dn and 2N littermates obtained from dams either treated with MCS or unsupplemented maternal choline (UMC) from pregnancy until weaning. Preliminary results display significant expression level differences based on genotype as well as by maternal diet. Classes of transcripts that show preliminary regulation by MCS include neurotrophins and neurotrophin receptors, synaptic-related markers, and protein phosphatases and kinases. In summary, these datasets will allow us to delineate expression changes associated with the Ts65Dn mouse model and to determine whether they can be mitigated by MCS. In addition, these findings will be compared to the previously reported patterns of gene expression in hippocampal CA1 pyramidal neurons as well as compared and contrasted to molecular fingerprints obtained from human postmortem brain tissues from subjects with DS and AD (along with age-matched non-demented controls) to assess potential translational targets.

Disclosures: **H.M. Chao:** None. **M.J. Alldred:** None. **S. Lee:** None. **E. Petkova:** None. **S.D. Ginsberg:** None.

Poster

681. Down Syndrome

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Support: Sie foundation postdoctoral award

Linda Crnic Institute seed Grant

MNIRGDP-12-258900

SFARI 274443

RO1-NS086933

NARSAD 21069

Title: Hippocampal network activity in a mouse model for Down syndrome

Authors: *G. J. LEVENGA[1,2], M. ROCHE[2], H. WONG[2], P. CAIN[2], C. A. HOEFFER[2,3]; [1]Dept. of Integrated Physiol., Univ. of Colorado Boulder, Boulder, CO; [2]Inst. for Behavioral Genet., Boulder, CO; [3]Dept. of Integrated Physiol., Boulder, CO

Abstract: Down syndrome (DS), also known as trisomy 21, is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21. Because of better education and improved health care, people with DS are living longer. With this increase in lifespan, it was found that people with DS develop symptoms consistent with early onset Alzheimer's disease (AD). Both AD and DS comorbid with abnormal EEG activity and seizures. In DS, seizures seem to peak at two ages; the first peak occurs in the first two years of age, while the second peak occurs during adulthood. The second peak may be connected with the symptoms of AD. One gene that is located on chromosome 21 called Regulator of Calcineurin 1 (RCAN1) may be involved in the development of hyperexcitability and other accelerated aging symptoms, such as oxidative stress and mitochondrial dysfunction. The goal of our research is to test the hypothesis that a) a mouse model of DS develops epilepsy during aging and b) RCAN1 is involved in DS/AD-related aging phenotypes and hyperexcitability. We use two different transgenic mouse models: Dp161Yey (Dp16) mice and RCAN1TG mice. Dp16 mice have a direct duplication of the entire Mmu16 region that is conserved in HSA21 and RCAN1TG mice only overexpress RCAN1 in excitatory neurons in the brain. To measure network excitability from freely behaving mice, we implanted electrodes in the hippocampus to measure local field potentials (LFPs). We

expect that young Dp16 mice have increased power in gamma frequencies due to excessive inhibition by the GABAergic circuit reported in DS model mice, while spiking frequency may be reduced. This phenotype might reverse in aged Dp16 mice where we expect to find increased hyperexcitability. Since RCAN1 is a major calcineurin (CaN) regulator and CaN KO mice show enhanced gamma frequency, we expect that RCAN1TG mice have similar phenotypes to Dp16 mice. In addition, we expect to find spontaneous epileptic events in aged Dp16 and RCAN1TG mice. This work will be important to better understand circuit dysfunction during aging in Down syndrome.

Disclosures: G.J. Levenga: None. **M. Roche:** None. **H. Wong:** None. **P. Cain:** None. **C.A. Hoeffer:** None.

Poster

681. Down Syndrome

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Topic: C.06. Developmental Disorders

Support: Cure Alzheimer's Foundation Grant 2014-3364.

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LuMind Foundation Grant 2015-0097

Title: Effects of monoacylglycerol lipase inhibitor JZL184 on adult neurogenesis in Ts65Dn mice, a model of Down syndrome

Authors: D. FOZOONMAYEH, M. SAWA, J. YU, A. BECKER, *A. M. KLESCHEVNIKOV;
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Abstract: Down syndrome (DS) is a developmental genetic disorder characterized by profound cognitive impairment. It was previously shown that one of the causes likely contributing to cognitive impairment in DS is impaired adult neurogenesis. Monoacylglycerol lipase (MAGL) is the key catabolic enzyme of endocannabinoid 2-arachidonoylglycerol (2-AG). Recently we observed that chronic treatment of Ts65Dn mice, a genetic model of DS, with the selective MAGL inhibitor JZL184 increased brain levels of 2-AG and improved hippocampal synaptic

plasticity and cognition. We hypothesized that one possible mechanism by which JZL184-treatment improved synaptic plasticity and cognition is through a restoration of adult neurogenesis. Here we examined the effects of chronic JZL184- treatment of Ts65Dn mice and their littermate WT controls on properties of adult neurogenesis in the dentate gyrus. Adult (8 mo old) male mice were injected with JZL184 (i.p., 8 mg/kg) once a day for 19 days. BrdU was injected once a day for 3 days (i.p., 100 mg/kg) during the chronic phase (days 12-14) of the JZL184 treatment. The brains were collected for immunohistochemistry 6 days after the last BrdU injection. In the vehicle-treated animals, density of BrdU-positive cells was almost three times lower in the Ts65Dn vs. WT mice (WT Veh: 100 ± 36%, Ts65Dn Veh: 33.4 ± 13.1 %). Treatment with JZL184 increased the density of BrdU-positive cells in Ts65Dn mice, but reduced it in WT controls. Expressed in percentage points of the corresponding vehicle-treated controls, the densities of BrdU-positive cells were significantly increased in the JZL184-treated Ts65Dn mice (WT JZL: 45.9 ± 10.3%; Ts65Dn JZL: 143.0 ± 14.1 %, $t(4) = 6.8$, $p = 0.001$). These data show that chronic MAGL inhibition improves neurogenesis in Ts65Dn, but impairs it in WT mice. Thus, restoration of the adult neurogenesis could underlie the improvement of synaptic plasticity and learning in JZL184-treated Ts65Dn mice. This result suggests that chronic MAGL inhibition should be considered as a potential approach for improvement of cognitive impairment in Down syndrome.

Disclosures: D. Fozoonmayeh: None. **M. Sawa:** None. **J. Yu:** None. **A. Becker:** None. **A.M. Kleschevnikov:** None.

Poster

681. Down Syndrome

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Topic: C.06. Developmental Disorders

Support: HD057564

AG043375

AG014449

AG017617

Alzheimer's Association IIRG-12-237253

Title: Effects of maternal choline supplementation (MCS) on neurotrophin and neurotrophin receptor expression in the Ts65Dn mouse model of Down syndrome and Alzheimer's disease

Authors: *S. D. GINSBERG[1,4,5], M. J. ALLDRED[1,4], I. ELAROVA[1], A. SALTZMAN[1], S. LEE[2], E. PETKOVA[3,6], B. E. POWERS[7,8], B. J. STRUPP[7,8], E. J. MUFSON[9,10];
[1]Ctr. for Dementia Res., [2]Med. Physics, [3]Child Psychiatry, Nathan Kline Inst., Orangeburg, NY; [4]Psychiatry, [5]Neurosci. & Physiol., [6]Child & Adolescent Psychiatry, New York Univ. Langone Med. Ctr., New York, NY; [7]Div. of Nutritional Sci., [8]Psychology, Cornell Univ., Ithaca, NY; [9]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [10]Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Persons with Down syndrome (DS) have intellectual disability and develop Alzheimer's disease (AD) neuropathology in early midlife. Currently, there are no effective treatments for DS or AD. A potential therapeutic strategy is dietary maternal choline supplementation (MCS). The Ts65Dn mouse model recapitulates key features of DS/AD, including cognitive dysfunction and basal forebrain cholinergic neuron (BFCN) degeneration, enabling mechanistic assessments that may be translated to humans. BFCNs are responsive to target-derived neurotrophic support from the hippocampus and neocortex, and MCS is posited to increase levels of nerve growth factor (NGF) and/or brain-derived neurotrophic factor (BDNF) and optimize their respective signaling pathways, which may underlie behavioral benefits seen in offspring from MCS treated dams. We hypothesize that MCS attenuates select neurotrophin and/or neurotrophin receptor changes associated with DS/AD. Here we evaluated homogenates of hippocampal and frontal cortex tissue obtained from offspring from MCS or choline unsupplemented mothers at 12-16 months of age. Immunoblot analysis was performed using antibodies directed against neurotrophins, including proNGF, proBDNF, and mature BDNF, as well as neurotrophin receptors including cognate NGF receptor TrkA, cognate BDNF receptor TrkB, cognate neurotrophin-3 (NTF3) receptor TrkC, and the pan-neurotrophin receptor p75NTR. Preliminary immunoblot analysis indicates a significant effect of maternal diet on offspring expression of proNGF and p75NTR in both hippocampus and frontal cortex. Specifically, MCS upregulates proNGF and p75NTR expression in both regions independent of genotype. Moreover, preliminary results indicate that TrkA expression is downregulated in Ts65Dn cortex compared to 2N littermates, and that MCS increases TrkA expression independent of genotype. These data suggest that MCS primes the brain of adult offspring for increased neurotrophic signaling, both through presumed pro-survival as well as pro-apoptotic pathways, effectively increasing the dynamic range of neurotrophic activity in the forebrain. Ongoing assessments of BDNF & TrkB and NTF3 & TrkC are predicted to show that MCS has a significant effect on the expression of these neurotrophic pathways as well. By understanding mechanisms underlying the beneficial effects of MCS, we may be able to increase the potential for translation to humans, and alterations in neurotrophic support from hippocampus and neocortex may be key areas for intervention in DS and AD.

Disclosures: **S.D. Ginsberg:** None. **M.J. Alldred:** None. **I. Elarova:** None. **A. Saltzman:** None. **S. Lee:** None. **E. Petkova:** None. **B.E. Powers:** None. **B.J. Strupp:** None. **E.J. Mufson:** None.

Poster

681. Down Syndrome

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 681.10/F44

Topic: C.06. Developmental Disorders

Support: NIH, NIDCD T32DC009401

Jerome Lejeune Foundation, Agreement 1326

Title: Lingual and laryngeal myosin heavy chain isoform characterization of the Ts65Dn mouse model of Down syndrome

Authors: ***T. J. GLASS**, N. P. CONNOR;
Surgery, Univ. of Wisconsin, Madison, WI

Abstract: Down syndrome is associated with a high incidence of impairment in vocal communication, feeding, and swallowing. The etiologies of these dysfunctions are unclear. The Ts65Dn mouse model was used to address the hypothesis that alterations in neuromuscular phenotypes underlie behavioral deficits in these critical cranial functions. Lingual and laryngeal muscles were isolated and analyzed by SDS-PAGE to quantify relative myosin heavy chain (MyHC) isoform composition. The following muscles were assayed: genioglossus (a tongue protrusor), sternohyoid (involved in maintaining airway patency), and anterior and posterior digastric (involved in jaw movement and positioning of the hyoid bone). Ts65Dn mice showed significant relative reduction in MyHC 2b in the digastric muscles ($n = 10$ adult mice per group, $p = .03$). Because relative MyHC 2b levels in the murine digastric muscles are known to increase following the developmental transition from weaning to mastication, studies are underway to assess digastric MyHC 2b levels at multiple points during post-natal development and to quantify functional mastication rates. Characterization of neuromuscular junctions in muscles pertinent to lingual and airway functions has also been initiated. In future work, analysis of the developmental trajectory of MyHC isoform changes in conjunction with analysis of neuromuscular junctions in the muscles of vocalization, mastication, and swallowing in Down syndrome mouse models may assist in a better understanding of the etiologies of vocal communication and feeding impairments associated with this syndrome.

Disclosures: **T.J. Glass:** None. **N.P. Connor:** None.

Poster

681. Down Syndrome

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Topic: C.06. Developmental Disorders

Support: NIH, NICHD/NIMH, RO1HD05780

Title: Absence of Down syndrome-related prenatal phenotypes in the Dp(16)1Yey/+ mouse model of Down syndrome

Authors: ***J. GOODLIFFE**, T. F. HAYDAR;
Boston Univ. Sch. of Med., Boston, MA

Abstract: Down syndrome (DS) is the most common genetic intellectual disorder and results in malformations of the central nervous system. Fetal studies have reported aberrations in brain developmental including microcephaly and altered cortical lamination. Several mouse models of DS have recapitulated these phenotypes as well as establishing further abnormalities in critical events including neurogenesis and neuronal differentiation. A recently developed model, the Dp(16)1Yey/+ or Dp16, has the largest triplication of the human chromosome 21 (Hsa-21) homologous region located to mouse chromosome 16 (Mmu16) and may better represent DS pathologies. To date, Dp16 studies have focused on adult behavioral, cerebellar and craniofacial abnormalities that mirror other mouse models of DS. Here, we present the first comprehensive study on Dp16 prenatal brain development. Despite the presence of adult phenotypes, our study shows that all measured parameters of Dp16 forebrain development are unchanged from euploid mice. Specifically, several phenotypes previously reported in human fetal neocortex and in the developing forebrains of other mouse models such as microcephaly, altered cortical lamination, reduced neurogenesis, progenitor population abnormalities and abnormal mitotic activity are not present in Dp16. This striking absence of DS-related phenotypes confounds the use of this model for embryonic studies and highlights the differences apparent in segmental models of trisomy 21. Data from this study isolate temporal periods belying observed deficits in the DS brain and for first time show that deficits in embryonic neurogenesis are not necessary for the manifestation of cognitive abnormalities in a mouse model of DS. Work supported by NIH, NICHD/NIMH, RO1HD05780

Disclosures: **J. Goodliffe:** None. **T.F. Haydar:** None.

Poster

681. Down Syndrome

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Topic: C.06. Developmental Disorders

Support: Fondecyt grant # 1130241 (Chile)

Title: Mechanism of synaptic vesicle exocytosis in neuronal cell lines derived from the cerebral cortex of and trisomy 16 fetal mouse, an animal model of Down syndrome: Regulation by cortical actin

Authors: J. VASQUEZ[1], A. CARDENAS[1], *P. A. CAVIEDES[2];
[1]Ctr. Interdisciplinario de Neurociencia de Valparaíso, Univ. de Valparaiso, Valparaiso, Chile;
[2]ICBM Fac Medicine, Univ. of Chile, Santiago, Chile

Abstract: Down Syndrome (DS) in man is produced by an extra copy of chromosome 21, and constitutes the most frequent cause of mental retardation for known genetic reasons. The trisomy 16 mouse is an animal model of DS, yet the condition is lethal in such animals, and they do not survive gestation. We have therefore generated cell lines from the cerebral cortex of a fetal Ts16 mouse (named CTb), and a normal littermate (named CNh), to use as control. Cultured brain neurons from TS16 fetuses, as well as the CTb cells, show a decrease in fractional release of [3H]-acetylcholine; compared to normal euploid controls. Therefore, we have explored the possibility that an alteration in the exocytosis of secretory organelles may be responsible for the decrease in acetylcholine (Ach) release. To evaluate this possibility, we used total internal reflection fluorescence microscopy (TIRF) to monitor exocytosis events in cultured CTb and CNh cells transfected with pHluorin fused to the vesicular acetylcholine transporter (VChAT). Our results reveal that, in resting conditions, CNh and CTb cells exhibit 5.6 ± 1.1 (n=37) and 10.1 ± 1.9 (n=26) exocytotic events in 3 min of recording, with fluorescence decay kinetics expressed in time constant values (τ) of 1.5 ± 0.2 and 1.9 ± 0.3 s respectively. The number of events significantly increase in cells stimulated with the Ca+2 ionophore ionomycin (20 μM), being such increment significantly lower in the CTb cells (22.7 ± 3.2, n=8) compared to CNh cells (39.4 ± 4.5, n= 10n= 8; $p<0.05$), while the decay kinetics decelerate similarly in both cell lines (τ of 2.0 ± 0.2 and 2.7 ± 0.3 s for CNh and CTb cells, respectively). Further, τ values increase when the cells are treated with 100 mM HEPES or 100 mM bafilomycin A1 (a vesicular ATPase inhibitor). Both agents delay vesicle acidification after closure of the fusion pore, yet they do not affect vesicles that fuse with the plasma membrane. Therefore, these results suggest that the main mechanism for release of vesicle content in these cell lines is a “kiss and run” type

exocytosis. On the other hand, after evaluating the role of the cytoskeleton in the exocytosis kinetics, we observed that the fluorescence decay kinetics is retarded in cells treated with the inhibitors of actin polymerization wiskostatin or cytochalasin D. Taken together, these results indicate that both cell lines release their vesicular content via a " kiss and run " mechanism that is highly regulated by the cortical actin cytoskeleton. The CTb cell line could represent an adequate model to study secretion impairments related to DS pathophysiology.

Disclosures: J. Vasquez: None. **A. Cardenas:** None. **P.A. Caviedes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent portection for CNh and CTb cell lines.

Poster

681. Down Syndrome

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Program#/Poster#: 681.13/G3

Topic: C.06. Developmental Disorders

Support: NIH Grant HD057564

RCPRS Funding

Title: Ts65Dn mice exhibit rapid forgetting in the social transmission of food preferences task: Further evidence for impaired hippocampal function

Authors: *N. A. SANTIAGO, S. E. KIM, B. E. POWERS, B. J. STRUPP;
Cornell Univ., Ithaca, NY

Abstract: Down syndrome (DS) is the most common genetic cause of intellectual disability, estimated to affect 1 in 690 live births. Individuals with DS also generally develop Alzheimer's disease (AD) pathology early in life, often including dementia. The Ts65Dn mouse model of DS recapitulates many of the hallmark characteristics of human DS, notably including cognitive dysfunction. Recent studies in our lab have demonstrated that increased maternal intake of choline during pregnancy and lactation in this mouse model significantly reduces cognitive dysfunction, increases hippocampal neurogenesis, and offers protection to cholinergic basal forebrain neurons. Prior to conducting additional experiments to further delineate the nature of the benefit offered by maternal choline supplementation in this model of DS, our goal was to first demonstrate impaired hippocampal function in these mice using a sensitive test of hippocampal function that does not involve food restriction or stress, factors which complicate

interpretation of observed performance impairment of this mouse model. One task with these characteristics is the Social Transmission of Food Preference Task. This task models the natural tendency of rodents to learn about the safety of novel foods through social interaction and has been shown to be sensitive to hippocampal dysfunction. In our first study, a 1 day memory retention interval was used, whereas our second study utilized a 7 day retention test. In the first study it was found that both genotypes exhibited strong social learning and recall one day later ($P< 0.001$). The trisomic mice did not significantly differ from their wildtype counterparts ($P= 0.417$). However, when a 7 day retention interval was used in the second study, group differences were uncovered. The mice overall demonstrated significant social learning and memory ($P= 0.0001$) with the interaction of social learning and genotype being borderline significant ($P= 0.06$), suggesting that the magnitude of the social learning effect varied by genotype. A very strong social learning effect was seen for the 2N mice ($P= 0.0001$), whereas it was not significant for the trisomics, indicating more rapid forgetting of the socially transmitted information. These results have laid the groundwork for using this task in future studies to test whether maternal choline supplementation will alleviate the impairment in hippocampal-dependent explicit memory seen in DS, and gain greater insight into underlying neural and epigenetic mechanisms.

Disclosures: **N.A. Santiago:** None. **S.E. Kim:** None. **B.E. Powers:** None. **B.J. Strupp:** None.

Poster

681. Down Syndrome

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 681.14/G4

Topic: C.06. Developmental Disorders

Support: NHMRC

Title: Elucidating the role of metals in the Down syndrome brain

Authors: *N. MALAKOOTI[1,2,3], M. A. PRITCHARD[2], R. C. KIM[3], I. T. LOTT[3], I. VOLITAKAS[1], B. R. ROBERTS[1], D. I. FINKELSTEIN[1], P. A. ADLARD[1];
[1]The Florey, Univ. of Melbourne, Parkville, Australia; [2]Biochem. and Mol. Biol., Monash Univ., Clayton, Australia; [3]Univ. of California, Irvine, CA

Abstract: Introduction: Down syndrome (DS) is the most common intellectual disability, with an incidence of 1 in 700 births and is caused by whole or partial trisomy of chromosome 21. All people with DS develop Alzheimer's disease (AD) like neuropathology by the age of 40. One of

the characteristics of AD is dyshomeostasis of metals in brain. Aim: Since one of the characteristics of AD is dyshomeostasis of metals in brain, we tested whether metal homeostasis was also altered in the DS brain. Method: We measured metal levels in the hippocampus, prefrontal and temporal cortices in nineteen post mortem DS brains (55.9 ± 7.2 years of age) and seven control brains (54.2± 3.07 years of age) by inductively coupled plasma mass spectrometry (ICPMS). Result: Iron levels were significantly higher and calcium levels were significantly lower in the hippocampus, prefrontal and temporal cortex in DS. Zinc levels were significantly lower in DS temporal cortex. Conclusion: These data suggest that metals are dysregulated in DS, The underlying cellular mechanisms of this failure in metal ion homeostasis remain to be explored and further interrogation of these samples with liquid chromatography ICPMS is currently underway to further characterize the metalloproteome in the DS brain.

Disclosures: N. Malakooti: None. **M.A. Pritchard:** None. **R.C. Kim:** None. **I.T. Lott:** None. **I. Volitakas:** None. **B.R. Roberts:** None. **D.I. Finkelstein:** None. **P.A. Adlard:** None.

Poster

681. Down Syndrome

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Topic: C.06. Developmental Disorders

Support: UCL Impact studentship

Title: Canonical wnt signalling alterations correlate with hsa21 trisomy and the kinase activity of dyrk1a

Authors: *S. GRANNO[1], D. BERWICK[2], F. WISEMAN[3], V. PLAGNOL[4], M. ZANDA[4], V. TYBULEWICZ[5], E. FISHER[3], K. HARVEY[2];
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Abstract: Down syndrome (DS), the most common human aneuploid disorder, is caused by trisomy of chromosome 21 (Hsa21). Recurring features include distinct morphological abnormalities and intellectual disability. In DS, overexpression of the Hsa21 gene dual specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (DYRK1A) is inextricably linked to the DS phenotype, as well as the nearly universal development of an early onset form of Alzheimer's disease (AD). The Wnt pathway is a highly conserved signalling cascade involved in transcriptional regulation of genes with recognised roles in cancer, stem cell differentiation

and neurogenesis. In recent years, aberrant Wnt signalling has been linked to development of AD. Several studies indicate that stabilisation of this signalling pathway may prevent Aβ-mediated neurotoxicity, while its disruption has been found to exert negative effects on synaptic assembly and function. Here, we seek to determine the role of Wnt signalling in the development of DS, combining studies in cell and animal model systems. Because of its documented interaction with the Wnt inhibitor GSK3β, we selected DYRK1A for primary investigation. In a neuroblastoma cell line (SH-SY5Y), we demonstrate DYRK1A's role as a Wnt signalling modulator through inhibition and overexpression studies. Furthermore, we show that DYRK1A overexpression leads to a significant alteration in protein amount of key Wnt signalling components. Taken together, these data suggest a novel modulatory role of DYRK1A on canonical Wnt signalling activity. Concurrently, our *ex vivo* studies on the Tc1 mouse model of DS seek to profile Wnt signalling in the DS cerebral cor-tex and hippocampus. Our results indicate that the Tc1 hippocampus and cortex display significant changes in expression levels of Wnt agonists, antagonists and negative modulators. Some of these are reportedly involved in hippocampal development and adult neurogenesis. Taken together, these results suggest an overall imbalance in canonical Wnt signalling in the Tc1 mouse model. Overall, our data indicate that aberrant Wnt signalling may significantly correlate with Hsa21 trisomy, both through DYRK1A-mediated modulation and changes in Wnt signaling component expression and activation levels. For the first time, Wnt signalling may be established as a key contributor to DS, opening new avenues for therapeutic strategies aimed at restoring physiological Wnt signaling function.

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Poster

681. Down Syndrome

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Support: AG043375

AG014449

AG017617

Alzheimer's Association IIRG-12-237253

Title: Maternal choline supplementation (MCS) alters CA1 pyramidal neuron gene expression in adult Ts65Dn and normal disomic (2N) offspring

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Abstract: Down syndrome (DS) is the most frequent genetic cause of intellectual disability (ID). Individuals with DS have decreased cognitive function seen by impairments in hippocampal learning and memory, degeneration of cholinergic basal forebrain neurons (BFCNs), and language and communication skills. In addition to abnormal CNS function during development and adult life, individuals with DS develop Alzheimer's disease (AD) neuropathological hallmarks early in mid-life, including senile plaques, neurofibrillary tangles, and early endosomal abnormalities. To examine gene expression changes associated with DS/AD and test a putative treatment paradigm, we are using the Ts65Dn mouse model. Ts65Dn mice recapitulate several critical components of DS/AD, including cognitive dysfunction and BFCN degeneration, providing mechanistic assessments for translation to humans. We tested a maternal choline supplementation (MCS) dietary paradigm and examined Ts65Dn and normal disomic (2N) offspring at the start of the BFCN degeneration (after ~4 months of age), postulating that MCS will improve cognition and could delay the septohippocampal degeneration seen in Ts65Dn mice, specifically by assessing CA1 pyramidal neurons. We tested whether MCS treatment could ameliorate some of the gene expression deficits found in Ts65Dn mice within the BFCN pathway and track pathways that may elucidate the underpinnings of cognitive decline and AD pathology. Microarray results on a custom-designed platform indicate that MCS produces significant gene expression level changes compared to age-matched unsupplemented maternal choline (UMC) offspring in CA1 pyramidal neurons both independent and dependent of genotype, which is confirmed by Nanostring nCounter and/or qPCR analysis. Specifically, alterations in several classes of transcripts including both glutamatergic neurotransmission and neurotrophin receptor activity, along with AD-related genes and synaptic-related markers were observed in Ts65Dn and 2N littermates. Preliminary genes that are MCS sensitive include App, Syp, Sod2, Ntf5, and Slc6a13. Comparing MCS offspring to UMC offspring in Ts65Dn and 2N littermates will help to elucidate specific genes and signaling pathways that may be responsive to this early intervention. Moreover, these mechanistic studies may help elucidate the link between choline requirements and cognitive development. This approach also has translational viability as a low cost, non-invasive method of cognitive improvement for DS, as well as having a generalized neuroprotective effect that could delay the onset and development of AD and related neurodegenerative disorders.

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Poster

681. Down Syndrome

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Support: Innovation Research Seed Award, Kent State University

Jérôme Lejeune Foundation

Title: Netrin-1 induces local translation of Down syndrome cell adhesion molecule in axonal growth cones

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Abstract: Down syndrome cell adhesion molecule (DSCAM) is known to play an important role in many neurodevelopmental processes such as axon guidance, dendrite arborization and synapse formation. *DSCAM* is located in the Down syndrome trisomic region of human chromosome 21 and implicated as one of the genes directly contributing to the Down syndrome brain phenotype, which includes a reduction in the formation of long-distance connectivity. Here, we find that overexpression of DSCAM in mouse cortical pyramidal neurons results in a decrease in axon outgrowth and branching. This finding directly implicates DSCAM as a contributor to the formation of improper neuronal connectivity in Down syndrome. Thus, it is of significant interest to understand the underlying molecular mechanisms by which *Dscam* regulates axon pathfinding. The local translation of a select group of mRNA transcripts within growth cones is necessary for the formation of appropriate neuronal connectivity. We have found that *Dscam* mRNA is localized to growth cones of C57BL/6J mouse hippocampal pyramidal neurons. Localization of *Dscam* mRNA to growth cones is dynamically regulated in response to the axon guidance molecule, netrin-1. Furthermore, netrin-1 stimulation results in an increase in locally translated DSCAM protein in growth cones. Interestingly, deleted in colorectal cancer (DCC), a netrin-1 receptor, is required for the netrin-1 induced increase in *Dscam* mRNA local translation. Locally translated mRNAs are transported in a translationally dormant state as a part of a ribonucleoprotein complex. We find that two RNA binding proteins, fragile X mental retardation protein (FMRP) and cytoplasmic polyadenylation element binding protein (CPEB), colocalize with *Dscam* mRNA in growth cones, suggesting their regulation of *Dscam* mRNA localization and translation. We are also examining these processes in a mouse model of Down syndrome (Ts65Dn), and have found that the formation of appropriate interhemispheric connectivity during

development is disrupted. Furthermore, the localization and local translation of *Dscam* mRNA in early postnatal neuronal growth cones from Down syndrome mice is also dysregulated. Taken together, these results have implications for Down syndrome, because dysregulated local translation of *Dscam* during embryonic development may contribute to inappropriate neural connectivity and the etiology of this neurodevelopmental disorder.

Disclosures: S. Jain: None. **K. Welshhans:** None.

Poster

682. Developmental Disorders: Angelman's

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Topic: C.06. Developmental Disorders

Support: Foundation for Angelman Syndrome Therapeutics

Title: A novel neurosteroid improves specific phenotypes of the Angelman Syndrome mouse model

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Abstract: Angelman Syndrome (AS) is a devastating neurological disorder for which there is no cure. AS presents with ataxia, frequent smiling and laughter, lack of speech, and severe, debilitating seizures that occur in >90% of the AS population. Epilepsy in AS is often refractory to many prescribed medications, and chronic, intractable epilepsy is shown to cause hippocampal damage and is associated with cognitive decline. The severity of seizures and lack of consistently effective anti-epileptic medications for AS patients demonstrates a considerable need for other therapeutic options. Decreases in GABAergic tone and cognition have been observed in the AS mouse model; the use of a GABA agonist lacking significant motor and memory-related side effects may dampen overall neuronal excitability and increase the signal-to-noise ratio in various areas of the brain, which could positively affect the motor, learning, and memory phenotypes. Thus, we examined a novel therapeutic strategy for seizures in AS: a novel neurosteroid with enhanced bioavailability. Ganaxolone, a partial agonist and positive allosteric modulator of GABAA receptors, is a novel synthetic analog of allopregnanolone, an endogenous neurosteroid. This drug has demonstrated success in clinical trials for complex partial seizures and pediatric

epilepsy with limited side effects. Therefore, we hypothesize that Ganaxolone will decrease seizure frequency, increase seizure threshold, and will improve hippocampal-dependent cognition and motor learning deficiencies seen in Ube3a-deficient mice. These mice were implanted with subcutaneous osmotic pumps for either 3 days or 4 weeks in order to evaluate both short- and long-term effects. We report that Ganaxolone improves specific phenotypes in the AS mouse model, and should be investigated further as a potential non-toxic therapy for individuals diagnosed with AS.

Disclosures: S.L. Blankenship: None. **J. Grieco:** None. **M. Rogawski:** None. **E. Weeber:** None.

Poster

682. Developmental Disorders: Angelman's

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Topic: C.06. Developmental Disorders

Support: R01MH085953

Title: Exploring alternative diffusion tensor measures to study atypical development in 22q11.2 deletion syndrome

Authors: *J. VILLALON REINA[1], J. GALVIS[1], C. CORBIN[1], T. NIR[1], L. KUSHAN[2], P. M. THOMPSON[1], C. BEARDEN[2];
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Abstract: Diffusion-weighted MRI (dMRI) is a widely used tool to study brain disease and can describe alterations in white matter microstructure in typical and atypical development. Fractional anisotropy (FA) is a standard dMRI-derived measure sensitive to disrupted white matter microstructure in neurodevelopmental disorders such as 22q11.2 deletion syndrome (22qDS). Many studies report higher FA in several brain regions in adolescents and children with 22qDS, relative to typically developing controls. Most studies of 22qDS patients acquire MRI scans with low angular resolution and lower b-values, which constrain the analysis to measures derived from Diffusion Tensor Imaging (DTI). We used alternative DTI derived metrics to further describe the microstructure of the areas with higher FA in 22qDS. We compared adolescents with 22qDS to typically developing control subjects (56 patients with molecularly confirmed 22q11.2 deletions; mean age=14.96 years, M/F 28/28, 52 controls; mean

age=12.33 years, M/F 31/21). MRI parameters were: 64 gradient directions, one shell of b=1000 s/mm2. Besides FA and mean diffusivity (MD), we also computed mode, linearity, sphericity, and planarity. These describe the geometry of the tensor providing an indirect measure the white matter architecture such as high dispersion of fibers vs. unidirectional single fiber compartments. We compared the two groups with a standard voxel-wise analysis. FA was higher in 22q in the body, isthmus and genu of the corpus callosum (CC) and in the corticospinal tract (CS), particularly on the left side. In the CC, the 22qDS group had higher linearity, lower sphericity and lower MD. In the CS, we found higher mode and lower planarity in the 22qDS group. In both the CC and CS, the tensors had a more elongated shape in 22q versus controls, so the higher FA may imply a less complex white matter architecture. Higher MD has been associated with changes in the extracellular space such as glial cell rearrangements. Even though DTI reconstructions with lower b-values cannot readily distinguish between extra- and intracellular compartments, Gaussianity is characteristic of the extracellular component of diffusion as is assumed in DTI. The higher FA in 22qDS may be caused by lower neurite density with a surrounding highly organized glial architecture along the main axis of the axonal pathways. More studies are required to distinguish between pathological changes of glial and axonal components, which may be facilitated by acquisitions with multiple diffusion-sensitive shells.

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Poster

682. Developmental Disorders: Angelman's

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Topic: C.06. Developmental Disorders

Support: Canadian Institue for Health Research

Ontario Brain Institute

Title: Assessing the neuroanatomy and behaviour in a mouse model of Angelman Syndrome revealed several sex differences

Authors: *J. ELLEGOOD[1], J. K. Y. LAI[2], K. C. RILETT[2], R. N. MACKENZIE[2], J. P. LERCH[1], J. A. FOSTER[2];
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Abstract: Background – Angelman Syndrome (AS) is a neurodevelopmental disorder caused by deletions in the chromosomal region 15q11-13. This region is susceptible to genetic imprinting, and therefore maternal and paternal deletions have differing affects, with a maternal deletion causing AS and a paternal deletion causing Prader-Willi Syndrome. Maternal loss of function in the gene *UBE3A*, which is found within 15q11-13, is sufficient to cause AS. *Ube3a*(p+/m-) mice have been previously investigated to study AS, and these studies have reported motor deficits and learning impairments. **Objectives** – The purpose of this study is to use MRI and several behavioural assays to investigate the neuroanatomical phenotype and how it relates to the behavioural phenotype in *Ube3a* (p+/m-) mice. **Methods** – In total 39 mice were assessed,18 mice were *Ube3a*(p+/m-) (10M and 8F) and 21 were wild-type (WT) (11M and 10F). Imaging was performed using a 7T MRI with a T2 weighted, 3D fast spin echo sequence which acquires data at an isotropic resolution of 56 μm (Lerch et al. 2011). Using image registration the brains were aligned, and the volumes of 62 different regions (Dorr et al. 2008) were calculated. Multiple comparisons were controlled using False Discovery Rate (FDR, Genovese et al. 2002). Prior to the *ex vivo* imaging the same group of mice were behaviourally tested. This testing consisted of measuring the righting reflex (at P4-6), USV recordings (P7), eye opening (P10-16), open field (P17), sociability (3 chamber task, P24), self-grooming (P25), and social interaction (P27). At P28 mice were perfusion fixed and prepared for ex-vivo imaging. **Results and Discussion** – For the full group of mice 17 regions were found to be smaller at an FDR of <5% (23 at FDR <15%). The female mice, however, drove those differences, where 17 regions were found to be smaller (FDR <15%). No differences found in the males. Figure 1 shows several coronal slices highlighting the significant voxel-wise differences for the full group. For the behavioural assays of the *Ube3a* mice, no differences found in righting reflex or weight, but there was a delay in eye-opening in both sexes. There was an increase in USV call duration in males as well as differences in call profiles in both sexes. At P17, males had decreased activity, whereas females were not different. Social and grooming behaviours were not different for either sex. **Conclusions** – Interestingly, sex differences were found in both the neuroanatomy and behaviour, but they seem to be inconsistent as the female neuroanatomy and the male behaviour were found to be more abnormal. Future investigation will examine any correlations between the neuroimaging and behaviour.

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Poster

682. Developmental Disorders: Angelman's

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Topic: C.06. Developmental Disorders

Support: Angelman Syndrome Foundation

Title: Role of Arc in plasticity deficits associated with Angelman syndrome

Authors: *E. D. PASTUZYN, J. D. SHEPHERD;
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Abstract: Angelman syndrome is an autism spectrum disorder that is caused by deletion of the maternal allele of the *UBE3A* gene, resulting in a lack of Ube3A protein in the brain. Previous studies have suggested that the neuronal protein Arc is a putative target of Ube3A ligase activity. Arc is an immediate-early gene that is rapidly transcribed in response to neuronal activity and is translated locally at synapses. Arc protein is critical for memory consolidation and multiple forms of synaptic plasticity via trafficking of AMPA receptors at the postsynaptic membrane. Arc levels are increased in the mouse model of Angelman syndrome, $Ube3a^{m-/p+}$ (AS mice), and reducing Arc levels in AS mice ameliorates seizure susceptibility. AS mice have impaired experience-dependent plasticity in visual cortex. Levels of synaptic proteins are tightly regulated; too much or too little protein at the synapse is detrimental for plasticity. Therefore, we hypothesize that increased Arc levels are causative in the plasticity deficits observed in AS mice. In order to verify a direct interaction between Arc and Ube3A protein *in vivo* and *in vitro*, we conducted coimmunoprecipitation experiments from whole brain lysates and from cultured cortical neurons. Our preliminary data suggest that Arc and Ube3A do not directly interact. However, we will also determine if this putative interaction is sensitive to both neuronal activity levels and spatial distribution of protein within neurons, as both Arc and Ube3A expression is activity-dependent. To test this, we will perform subcellular fractionation on whole brain lysates and coimmunoprecipitate Arc and Ube3A to determine if there is an interaction between these two proteins only in specific cellular compartments, such as in the nucleus or at synapses. In addition, we cultured cortical neurons from AS mice and wildtype littermates and performed immunohistochemistry for Arc in conditions of low or high neuronal activity. We found that the increase in Arc protein in AS mouse neurons is exaggerated by enhancing neuronal activity. Ongoing experiments will determine whether surface expression and trafficking dynamics of AMPA receptors are disrupted in AS mouse neurons. These studies will shed light on the putative Arc/Ube3A interaction and whether dysregulation of Arc in AS mice leads to AMPA receptor trafficking and plasticity deficits.

Disclosures: E.D. Pastuzyn: None. **J.D. Shepherd:** None.

Poster

682. Developmental Disorders: Angelman's

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Topic: C.06. Developmental Disorders

Support: NIH Grant P01NICHD033113

Title: Defining the phenotype: increases in neuronal density and glia-to-neuron ratio in the pre-commissural caudate head in Williams syndrome

Authors: *K. HANSON[1], C. N. BROWN[2], V. A. JUDD[1], H. A. ORFANT[1], U. BELLUGI[3], K. SEMENDEFERI[1];
[1]Anthrop., UCSD, La Jolla, CA; [2]Psychological & Brain Sci., UCSB, Santa Barbara, CA; [3]Salk Inst. for Biol. Res., La Jolla, CA

Abstract: Williams syndrome is a rare neurodevelopmental disorder caused by a hemizygous deletion of 26-28 genes on the seventh chromosome. Among its most notable behavioral phenotypes is a generalized disinhibition of social behavior. A significant decrease in activation is reported in functional imaging studies in frontostriatal regions during go/no-go tasks in individuals with Williams syndrome, with particular deficits in striatal activation. Given these findings in Williams syndrome, and the specific impairment of the caudate nucleus in a wide variety of other neurodevelopmental disorders affecting behavioral control and response inhibition, such as Huntington's disease and ADHD, we sought to examine the head of the caudate nucleus in individuals with Williams syndrome. Our sample included four pairs of age, sex, and hemisphere-matched subjects, including three males and one female (ages 18-45) who prior to death were part of an ongoing study seeking to characterize the behavioral, social, and cognitive effects of Williams syndrome. Coronal sections through the rostral portion of the caudate nucleus, consisting of the pre-commissural head of the caudate, were Nissl stained to examine the distribution of neurons and glia using unbiased stereological methods. We found an overall increase in neuronal density, as well as an increase in the ratio of glial cells to neurons. These data suggest that deficits in inhibitory behavioral control may be linked to dysfunction of local circuitry within the striatum in Willliams syndrome mediated by imbalance between neuronal and glial cell distribution and increased cell packing density. Control specimens and ongoing material support has been provided by the University of Maryland Brain and Tissue Bank.

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Poster

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Topic: C.06. Developmental Disorders

Support: NIMH RO1 29032

ENIGMA U54

Title: Using machine learning to identify a cortical neuroanatomic signature of 22q11.2 Deletion Syndrome

Authors: D. SUN[1], R. JONAS[1], E. KRIKORIAN[1], L. KUSHAN[1], M. GUDBRANDSEN[2], E. DALY[2], C. ECKER[2], C. MURPHY[2], D. MURPHY[2], M. CRAIG[2], *C. E. BEARDEN[1]; [1]UCLA, Los Angeles, CA; [2]King's Col. London, London, United Kingdom

Abstract: 22q11.2 Deletion Syndrome (22q11DS) is a neurogenetic disorder caused by a microdeletion of chromosome 22. Patients with 22q11DS exhibit elevated rates of psychiatric disorders including schizophrenia, autism, and ADHD. Thus, studying neuroanatomic changes in 22q11DS may shed light on neural mechanisms underlying the risk for developing psychiatric conditions. Marked brain abnormalities have been found in 22q11DS, but whether they constitute distinct patterns to differentiate patients from healthy individuals is unclear. With a relatively large magnetic resonance imagining (MRI) dataset, we seek to determine if 22q11DS patients can be classified based on cortical anatomical features using machine learning algorithms. MRI scans of 80 22q11DS patients (mean age 14.5; 48.5% female) and 93 demographically comparable healthy controls obtained from two research sites were included in the analysis. Anatomical measures including cortical thickness (CT), surface area (SA), and volume in 68 regions across the cortex were extracted using FreeSurfer's automatic parcellation method. Between-group comparisons were conducted, and Bonferroni correction was used to control multiple comparisons. Classification was done by applying a linear support vector machines algorithm to all cortical measures. Compared with controls, significantly reduced cortical volume was found in 22q11DS patients in parietal, occipital, and temporal regions, as well as in the anterior cingulate. Significantly increased CT was found in patients in the bilateral insula, left pars triangularis and opercularis, and right supramarginal and rostral middle frontal region, while significantly reduced CT was found in patients in the left parahippocampal region. Significantly reduced SA was widespread in patients, found in 45 regions across the cortex. Classification using within-group cross-validations on 70 patients and 57 controls from one site gave an average accuracy of 91.0% (87.1-96.8%). When the classifier derived from one site was applied to cortical measures of 10 patients and 26 controls from the other site, all except one

control were accurately classified, giving a cross-site classification accuracy of 97.2%. This study reveals profound anatomic abnormalities in the cerebral cortex of patients with 22q11DS, involving widespread reduction of cortical surface area and volume in primarily posterior brain regions, with relative preservation of frontal regions. These patterns allow highly accurate classification of patients and controls. Future studies may elucidate the role of these patterns in increasing the risk for 22q11DS-related psychiatric disorders.

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Poster

682. Developmental Disorders: Angelman's

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Support: Aim for the Top University Plan, National Chiao Tung University and Ministry of Education, Taiwan

Ministry of Science and Technology, Taiwan, MOST 103-2221-E-039-007-MY3

Title: Go/NoGo EEG experiment reveals cortical slowing in motor initiation in the children with cerebral palsy

Authors: C.-Y. LI[1], C.-H. CHANG[1], C.-Y. PENG[2], *J.-R. DUANN[3,4];
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Abstract: Introduction: The study of EEG characteristics of the children with cerebral palsy (CP) has been limited. In this study, we explored the EEG characteristics of the children with CP by finding the P300 evoked by the performance of a Go/NoGo task and comparing it to that of normal subjects. We hypothesized that the slowing in the task performance of the children with CP might be manifested by the slowing in the cortical processes caused by the damage to the motor-related cortices in the brain. As a result, we should see the changes in the shape of the P300s facilitated by the Go/NoGo task performance. The potential alterations included either the overall delay of P300 or the reshaping of the P300 subcomponents (Turetsky et al., 1998), indicating the slowing in perception or the cortical slowing in the motor initiation. Methods: We

collected EEG data from 20 children with CP, performing a visual Go/NoGo task, pressing a red huge round button using their dominant hand when they saw a circle on the screen and holding the button press when they saw a cross. Each visual cue was displayed for 500 ms at the inter-stimulus interval of 3 sec on average. 50 Go and 50 NoGo trials were collected in each EEG run and each subject performed two EEG runs. EEG data were recorded using a wireless 32-channel device with International 10-20 system cap using wet EEG electrodes. Each channel was sampled at 2 KHz and then filtered by a band-pass filter (1 - 50 Hz) and downsampled to 250 Hz for further analysis. We then decomposed the brain EEG into independent sources using independent component analysis and selected the brain EEG activity mainly locating at posterior midline regions for computing the event-related potentials (Jung et al., 2000). Results: The preliminary result showed that the P300 elicited by the Go/NoGo task performance of the children with CP revealed a clear gap between the subcomponents of P300 as compared to the P300 waveforms obtained from a Go/NoGo task performance by normal subjects reported in the literature. However, the early component of the P300 ERP arrived no later than the P300 found in the normal subject. Conclusion: Our results showed that the slowing in the task performance of the children with CP might be caused by the initiation of the motor action in the brain, while the capability of perception of the execution cues in their brain may be relatively intact as. References: Jung TP, et al., Removing electroencephalographic artifacts by blind source separation, Psychophysiol 37: 163-178, 2000. Turetsky BL, et al., P300 subcomponent abnormalities in schizophrenia: Physiological evidence for gender and subtype specific differences in regional pathology, Biol Psychiatry 43: 84-96, 1998.

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Poster

682. Developmental Disorders: Angelman's

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Title: Morphological analysis of TSC-1 deleted pyramidal neurons

Authors: K. DALEEN[1], T. MANGOUBI[3], R. COX[3], *A. YOSHII[2];
[2]Dept of Anat. & Cell Biol., [1]UIC, Chicago, IL; [3]MIT, Cambridge, MA

Abstract: Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disorder that causes abnormal cellular proliferation, differentiation, and growth in many organ systems including the brain, eyes, heart, lung, liver, kidney, and skin. Afflicted patients have a variety of neurological symptoms such as epilepsy, mental retardation and autistic behavior. Formation of cortical tubers, malformed tissues in the brain, accounts for some forms of seizures in those with TSC. However, it remains unclear whether tubers cause cognitive deficits. It is also possible that cortical neurons themselves are disorganized and that their synapse formation is dysregulated. In this study we analyze neuronal morphology in TSC. Using *in utero* electropolation, we transferred a DNA construct encoding GFP tagged Cre recombinase into embryonic day 15.5 neuronal progenitor cells in a Tsc1loxp/loxp mouse fetal brain. We imaged pyramidal neurons at postnatal day 28-30 with a confocal microscope. In WT cortices, Cre-GFP positive neurons were located in cortical layer 2/3. However, mutant brains showed a scattered distribution of Cre-GFP positive neurons. These Tsc-1 deleted cells also expressed a marker protein for layer 2/3 despite their malpositions. Furthermore, we found that soma size of Tsc1-deleted pyramidal neurons was enlarged as compared with WT pyramidal neurons. Sholl analysis demonstrated that Tsc1-deleted neurons had more arborizations than WT. We are currently analyzing dendritic spine size and number per a defined length. Our ongoing study suggests that Tsc-1 deleted cortical neurons show alterations in cellular organization and differentiation. In the future, we will aim to correlate these morphological changes with the neurological deficits in TSC. DoD TSCRP Career Transition Award (W81XWH-09-1-0088) to A.Y.

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Poster

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Topic: C.06. Developmental Disorders

Support: Foundation of Angelman Syndrome Therapeutics

Title: Spontaneous epileptiform activity and low seizure threshold in a mouse model of Angelman Syndrome

Authors: ***A. DAO**, H. A. BORN, A. ANDERSON;
Pediatrics Neurol., Baylor Col. of Med., Houston, TX

Abstract: Angelman Syndrome (AS) is a rare neurodevelopmental disorder that affects approximately 1 in 20,000 births. In addition to severe cognitive deficits, epileptic seizures are common in AS cases. These seizures are often refractory to standard anti-epileptic drug treatment. Thus, there is a need to characterize the seizure phenotype and threshold to convulsant to then use to screen novel therapeutics. We investigated the seizure phenotype in a mouse model of AS via deletion of exon 2 on chromosome 15. We utilized long-term video synchronized electroencephalography (v-EEG) and spectral analysis to qualitatively and quantitatively compare between wild-type (WT) and AS mice. We observed spontaneous spike bursting (polyspikes) in the cortex and hippocampus of AS mice on the C57BL/6 background, which correlated with a higher delta power in these regions and higher theta and gamma power in the hippocampus. These findings were not observed in the 129Sv/Ev background mice. We did not capture spontaneous seizures in long-term v-EEG recordings of AS mice in either C57BL/6 or 129Sv/Ev background, so we evaluated seizure threshold in these mice using kainate, a chemoconvulsant. We found that 129 Sv/Ev AS mice showed a lower seizure threshold compared to WT littermates when challenged with kainate, however a seizure threshold comparable to WT littermates was found in the C57BL/6 AS mice. These preliminary findings indicate strain specificity for spontaneous epileptiform activity, spectral changes, and decreased seizure threshold, and provide the fundamental groundwork for further studies into treatment of the critical seizure phenotype in AS.

Disclosures: **A. Dao:** None. **H.A. Born:** None. **A. Anderson:** None.

Poster

682. Developmental Disorders: Angelman's

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 682.10/G17

Topic: C.06. Developmental Disorders

Title: Motor control during dual-task situations in Williams syndrome

Authors: *K. WUEBBENHORST[1], N. HOLL[2];
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Abstract: Human motor functions comprise the planning, the realization and the control of movements. The motor functions of patients with Williams-Beuren Syndrome underlie identical mechanisms like their peers. Nevertheless, several alterations in movement execution are existent. Differences in motor execution restrain their everyday life in different ways. The origin of these differences is diverse since there is not one, but many causes for motor deficits. In

particular, difficulties in tasks with motor and cognitive requirements like climbing stairs are attributed to functional deficits in visualspatial processing (Meyer-Lindenberg et al., 2006). However, the often occurring change from hypotonic to hypertonic muscle tone during adolescence can be caused from deficits in processing motor commands. This leads to the high importance of the motor development and the necessity of its comprehensive and long-term promotion. In this study 27 subjects (*) between the ages from 7 to 30 participated for the evaluation of static postural control. We organized the participants in three age groups for better within group comparisons (age group I children 7-13, age group II adolescents 14-17, age group III young adults 18-24). Since patients constitute deficits in visuospatial planning of movements (Hocking et al., 2013) we introduced a motor-cognitive dual-task-situation in addition to a single-task-situation. The measurements of the static postural control were performed on a balance coordination system (GKS-1000, Meditech, Germany). The area represents the surface covered by the displacement of the body's center of gravity. The medial-lateral-velocity (ML-velocity) and the anterior-posterior-velocity (AP-velocity) represent the mean displacement velocity of the body's center of gravity when travelled at equal measuring time. The area increased for every age group when compared with the single-task and dual-task-situation. We found the highest increase with age group I from 5.1 ± 1.7 SE to 9.6 ± 3.2 SE for the dual-task-situation ($p=0.01$). The same results were obtained for the ML-velocity and AP-velocity. For instance, we found an increase of the ML-velocity in age group I from 14.7 ± 1.7 SE to 21.5 ± 2.8 SE ($p<0.001$). These results indicate that when confronted with a motor-cognitive dual-task-situation, the motor task is compromised for the cognitive task. Following these results, training of visualspatial perception along with a combination of sensorimotor training with cognitive involvement might improve the observed deficits in task execution. * matched healthy control subjects are recruited and data will be available for the Neuroscience congress

Disclosures: K. Wuebbenhorst: None. **N. Holl:** None.

Poster

682. Developmental Disorders: Angelman's

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 682.11/G18

Topic: C.06. Developmental Disorders

Support: Foundation for Angelman Syndrome Therapeutics Integrative Research Environment

Title: Strain-specific behavioral deficits in the maternally deficient Ube3a Angelman syndrome mouse model

Authors: *H. A. BORN[1,3], A. T. DAO[1,3], A. E. ANDERSON[2,3];
[1]Pediatrics, [2]Pediatrics, Neurology, and Neurosci., Baylor Col. of Med., Houston, TX; [3]Jan and Dan Duncan Neurolog. Res. Inst., Texas Children's Hosp., Houston, TX

Abstract: Angelman syndrome (AS) is a neurodevelopmental disorder characterized by marked developmental delay, cognitive impairment, movement disorder, and frequently is associated with seizures and abnormal EEG activity. AS is caused by defects in the maternally inherited ubiquitin protein ligase (Ube3a) gene, and a Ube3a maternal deficient mouse model (Ube3am-/p+) is a useful tool for AS therapeutic development. Despite the severity of AS, there is currently no effective treatment. To screen potential therapeutics, we established a behavioral battery and characterized the Ube3am-/p+ model on three different mouse strain backgrounds: C57Bl/6 (C57), 129Sv/Ev (129), and the F1 hybrid of C57 x 129. Previous studies have shown varying behavioral results, and one potential confound is differences from strain background. We assessed motor activity, anxiety, and learning and memory behavior in adult gender-balanced AS and wildtype (WT) littermates from C57, 129, or F1 hybrid background. The battery included open field activity (OFA), elevated plus maze (EPM), marble burying (MARB), inverted grid (IG), wire hang (WH), accelerating rotorod (RR), novel object recognition (NOR), and tone-shock paired fear conditioning (FC). Regardless of strain, AS mice show significantly increased weight and normal performance on the IG test. On a C57 background, AS mice show significantly decreased locomotor activity, rearing episodes, performance on both wire hang and rotorod, marbles buried, and preference for the novel object with a 24 hr test. In contrast, mice on a 129 background show normal locomotor activity, performance on the rotorod, and marble burying when compared to WT. FC-context and wire hang tests reveal deficits in AS 129 mice compared to WT 129 mice. The F1 hybrid AS mice show significantly decreased locomotor activity, rearing episodes, performance on rotorod, and marbles buried. Despite the impaired performance on motor tests of the AS F1 mice, preliminary results show similar performance from AS and WT F1 mice on NOR and FC tests. Our results suggest that AS-like behavioral phenotypes expressed in the Ube3am-/p+ mouse model are strain-specific. The Wt 129 mice perform poorly on the OFA, MARB, and NOR tests, suggesting a 129 background may not be optimal for screening the effect of potential AS treatments on both motor and learning/memory impairments. Overall, the C57 background will be best for screening using a diverse battery of behavioral tests. Future studies will screen potential treatments for rescue of AS-like motor and learning and memory impairments. Support: Foundation for Angelman Syndrome Therapeutics Integrative Research Environment

Disclosures: H.A. Born: None. **A.T. Dao:** None. **A.E. Anderson:** None.

Poster

682. Developmental Disorders: Angelman's

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 682.12/G19

Topic: C.06. Developmental Disorders

Support: Heinz C. Prechter Bipolar Research Fund

Title: Dorsal-ventral patterning in a neurodevelopmental model of bipolar disorder

Authors: *M. BAME[1], C. J. DELONG[2], M. G. MCINNIS[3], K. S. O'SHEA[2];
[2]Cell & Developmental Biol., [3]Psychiatry, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Bipolar Disorder is a hereditary neuropsychiatric condition in which patients experience intense mood fluctuations that can lead to extreme behavioral changes. Moods alternate between severe depression and mania and often negatively impact patients' lives. No single gene has been determined to cause Bipolar disorder and the underlying pathological mechanisms remain unclear. It has recently been suggested that disruptions in neural development may play a role in the pathology of psychiatric disorders. To test this hypothesis, we have established a developmental model of Bipolar Disorder in which patient and control somatic cells have been reprogrammed, establishing induced pluripotent stem cells that have been differentiated into neural precursors and neurons. Microarray analysis of RNAs from these cells indicate that neurons derived from Bipolar patients have elevated expression of transcription factors associated with GABAergic interneurons, while those derived from controls have elevated expression of transcription factors associated with excitatory glutamatergic neurons. To examine the mechanisms involved in the production of ventral CNS derivatives (GABAergic interneurons) and dorsal derivatives (excitatory glutamatergic neurons) we have exposed cells at sequential stages of differentiation to Hedgehog pathway agonists and inhibitors and to Wnt pathway stimulation. We found that when treated with an activator of Hedgehog pathway signaling (SAG), neural precursors derived from Bipolar patients respond more strongly than those derived from control patients, increasing expression of GLI1 and PTCH 1 transcripts, as well as the interneuron progenitor transcription factor NKX2.1. We are currently examining the response of neural precursors to Chir99021 (a Wnt pathway activator) and cyclopamine (a Hedgehog inhibitor) on expression of Hedgehog and Wnt target genes. Alterations in early neuronal allocation/differentiation could lead to alterations in patterning and connectivity throughout the cortex and contribute to Bipolar Disorder.

Disclosures: M. Bame: None. **C.J. DeLong:** None. **M.G. McInnis:** None. **K.S. O'Shea:** 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.; Genetech, Inc..

Poster

682. Developmental Disorders: Angelman's

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 682.13/G20

Topic: C.06. Developmental Disorders

Support: NIH MH57014

NIH MH104158

NIH NR012688

DARPA HR0011-14-1-0001

DARPA HR0011-12-1-0015

Pitt-Hopkins Research Foundation

Civitan International

Title: The basic neurobiology of Pitt-Hopkins Syndrome

Authors: *A. J. KENNEDY, J. J. DAY, D. SWEATT;
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Abstract: Human haploinsufficiency of the transcription factor Tcf4 (Transcription factor 4, E2-2, ITF2) leads to a rare autism spectrum disorder called Pitt-Hopkins syndrome (PTHS), which is associated with severe language impairment and development delay. However, the neural and transcriptional mechanisms disrupted in Tcf4 deficiency are unknown. Here, we demonstrate that Tcf4 haploinsufficient mice that genetically model PTHS have deficits in social interaction, ultrasonic vocalization, prepulse inhibition, as well as spatial and associative learning and memory. We found that despite learning deficits, Tcf4 (+/-) mice have enhanced long-term potentiation in the CA1 area of the hippocampus. Tcf4 was found to regulate hippocampal CpG methylation of gene bodies and cAMP response elements broadly across the genome, and direct the expression of neuroreceptors and memory associated genes, including Grin2a, Klotho, and Arc. In translationally oriented studies we found that small molecule HDAC inhibitors normalized hippocampal LTP and memory recall in our mouse model. Finally, we observed that antisense oligonucleotide-based HDAC2 isoform-selective knockdown was sufficient to rescue deficits in spatial and associative memory in Tcf4 (+/-) mice.

Disclosures: **A.J. Kennedy:** None. **J.J. Day:** None. **D. Sweatt:** None.

Poster

682. Developmental Disorders: Angelman's

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 682.14/G21

Topic: C.06. Developmental Disorders

Support: R01 NS056244

Australian NHMRC Senior Principal Research Fellowship 1041920

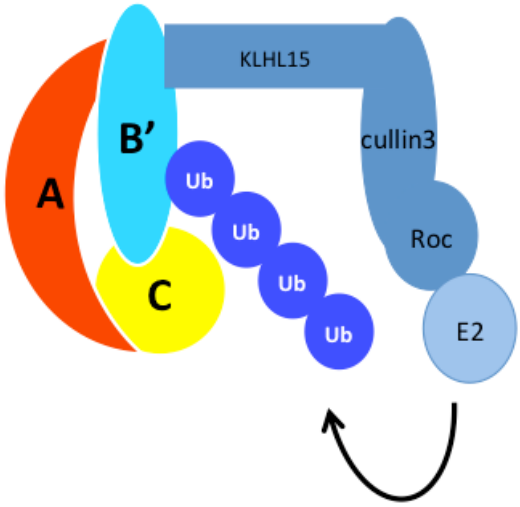
NHMRC Program Grant 628952

Title: Molecular characterization of KLHL15 mutations that cause intellectual disability

Authors: *J. SONG[1], R. MERRILL[1], M. SHAW[2,3], R. CARROLL[2,3], V. KALSCHEUER[4], F. MCKENZIE[5], L. JOLLY[2,3], J. GÉCZ[2,3], S. STRACK[1];
[1]Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA; [2]Robinson Inst., [3]Sch. of Paediatrics and Reproductive Hlth., The Univ. of Adelaide, Adelaide, Australia; [4]Dept. Human Mol. Genet., Max Planck Inst. for Mol. Genet., Berlin, Germany; [5]Genet. Services of Western Australia, Perth, Australia

Abstract: There is a ~30% excess of males with intellectual disability (ID), which can be partly explained by above average, 5-10%, contribution of genes and mutations on the X-chromosome. By X-exome sequencing, we identified mutations in the KLHL15 gene that cause ID in two Australian families. The first mutation deletes two poorly conserved amino acids (F241, Y242) in the middle of the protein, while KLHL15::ACOT9 is the result of a large in-frame chromosomal deletion, fusing the N-terminus of KLHL15 to the C-terminus of the mitochondrial enzyme acyl-CoA thioesterase 9. KLHL15, or Kelch-like 15, is the substrate binding component of an E3 ubiquitin ligase complex that also contains cullin3 (Cul3). We have previously shown that KLHL15 specifically targets the B′β (B56/PR61/PPP2R5B) subunit of protein phosphatase 2A (PP2A) for ubiquitylation and proteasomal degradation. B′β is a brain-specific regulatory subunit and mediates the dephosphorylation and inactivation of tyrosine hydroxylase and Ca^{2+}/calmodulin-dependent protein kinase II. As a first step towards elucidating pathogenic mechanism in XLID caused by mutations in E3 ligases, we have begun to biochemically characterize KLHL15 ΔFY241 and KLHL15::ACOT9. KLHL15 mutant cDNAs were cloned and characterized by expression in HeLa and HEK293 cells. The two XLID mutations did not alter the cytosolic localization of KLHL15, nor did they compromise binding to Cul3 as detected by co-immunoprecipitation. However, both ΔFY241 and ::ACOT9 dramatically decreased KLHL15 binding to PP2A/B′β, 35% and 17% , respectively, in comparison to wild-type

KLHL15. In addition, both XLID mutations impaired PP2A/B′β degradation and KLHL15 auto-degradation. Our results indicate that XLID mutations in KLHL15 are hypomorphic and suggest that regulated turn-over of PP2A/B' β and perhaps other as yet undiscovered substrates of KLHL15 is critical for proper development of the human brain.



Disclosures: **J. Song:** None. **R. Merrill:** None. **M. Shaw:** None. **R. Carroll:** None. **V. Kalscheuer:** None. **F. McKenzie:** None. **L. Jolly:** None. **J. Gécz:** None. **S. Strack:** None.

Poster

682. Developmental Disorders: Angelman's

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 682.15/G22

Topic: B.08. Synaptic Plasticity

Support: Neuro-Bsik

Title: Enhanced transmission at the calyx of Held synapse in a mouse model for Angelman syndrome

Authors: ***T. WANG**, Y. ELGERSMA, J. G. G. BORST;
Erasmus Med. Ctr., Rotterdam, Netherlands

Abstract: The neurodevelopmental disorder Angelman syndrome (AS) is characterized by intellectual disability, motor dysfunction, distinct behavioral aspects and epilepsy. AS is caused by a loss of the maternally expressed *UBE3A* gene, and many symptoms can also be observed in

an *Ube3a* mouse model of this syndrome. At the cellular level, changes in the axonal initial segment (AIS) have been reported, and changes in vesicle cycling have indicated the presence of presynaptic deficits. However, the physiological consequences of these changes for synaptic transmission *in vivo* are unknown. Here we studied the role of UBE3A in the auditory system, a pathway which is uniquely suited to study presynaptic function *in vivo*, by recording synaptic transmission at the calyx of Held synapse in the medial nucleus of the trapezoid body (MNTB). The calyx of Held synapse has a relay function in the auditory brainstem; each principal MNTB neuron is contacted by a single, giant terminal from a globular bushy cell of the contralateral AVCN. Because of its large size, both pre- and postsynaptic activity can be recorded *in vivo* with a single pipette. Through *in vivo* whole cell and juxtacellular recordings in *Ube3a* mice, we show that MNTB principal neurons exhibit a hyperpolarized resting membrane potential, an increased action potential (AP) amplitude and a decreased AP halfwidth. Moreover, both the pre- and postsynaptic action potentials in the calyx of Held synapse of AS mice showed significantly faster recovery from spike depression. An increase in AIS length was observed in the principal MNTB neurons of AS mice, providing a possible substrate for these gain-of-function changes. In addition, EPSPs showed decreased short-term synaptic depression (STD) during long sound stimulations in AS mice, and faster recovery from STD following these tones. These findings provide the first *in vivo* evidence for abnormal excitability and changes in synaptic transmission in a mouse model of AS.

Disclosures: **T. Wang:** None. **Y. Elgersma:** None. **J.G.G. Borst:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.01/G23

Topic: C.06. Developmental Disorders

Support: Baylor College of Medicine IDDRC Grant Number 1 U54 HD083092 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development

Title: The cognitive and behavioral phenotypes of individuals with CHRNA7 duplications

Authors: *M. GILLENTINE[1], C. P. SCHAAF[1,2];
[1]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [2]Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX

Abstract: Chromosome 15q13 is one of the least stable regions in the genome, due to the presence of low copy repeat elements (LCRs) that cluster into six breakpoints (BP1 to BP6). These LCRs make the region vulnerable to non-allelic homologous recombination (NAHR), resulting in recurrent copy number variants (CNVs). Between BP4 and BP5, multiple recurrent CNVs have been observed. Large deletions of 1.5 Mb to 2 Mb have been well established as pathogenic, with high penetrance of multiple neuropsychiatric phenotypes observed, including intellectual disability (ID), developmental delay (DD), autism spectrum disorder (ASD), schizophrenia, and epilepsy. Smaller deletions encompassing only *CHRNA7* have also been seen in individuals with a similar range of phenotypes and penetrance. However, the significance of duplications spanning *CHRNA7* has been enigmatic, as gains of *CHRNA7* have been found to occur at similar frequencies in individuals with neuropsychiatric disease and controls. Using a genotype-to-phenotype approach, we evaluated 18 individuals, who had been identified to carry a *CHRNA7* duplication by clinical chromosome microarray analysis. Duplication sizes varied from 148 kb to 3.231 Mb. Formal cognitive assessment of the 18 probands (age 2 to 14) revealed an average full scale IQ of 80.2, being significantly lower than the average population. Research reliable testing for autism spectrum disorder was positive in 41% of cases, as determined by the Autism Diagnostic Interview –Revised (ADIR) and Autism Diagnostic Observation Schedule (ADOS). Additional phenotypes included developmental delay, language and speech delay, attention deficit/hyperactivity disorder (ADHD), and epilepsy in a subset of patients. Inheritance was determined in 13 of 18 cases, with just over half of the transmitting parents manifesting a neurological phenotype, indicating that these duplications are incompletely penetrant. This study suggests that *CHRNA7* duplications may be pathogenic, and that affected individuals manifest cognitive and behavioral phenotypes. However, ascertainment bias needs to be considered in this context. A detailed neuropsychological phenotyping of individuals who were not referred as probands, but subsequently identified to carry a *CHRNA7* duplication, or individuals with a prenatal diagnosis of *CHRNA7* duplication could be considered to overcome that bias.

Disclosures: **M. Gillentine:** None. **C.P. Schaaf:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.02/G24

Topic: C.06. Developmental Disorders

Support: P01HD057853

NSF 1144399

Title: Behavioral and neuroanatomical characterization of mice with a mutation of the candidate dyslexia susceptibility gene Dyx1c1

Authors: *A. R. RENDALL[1], A. TARKAR[2], H. M. CONTRERAS-MORA[1], J. LOTURCO[2], R. H. FITCH[1];
[1]Psychology, [2]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: Dyslexia is a learning disability characterized by difficulty learning to read and write. The underlying biological and genetic etiology remains poorly understood. One candidate gene, dyslexia susceptibility 1 candidate 1 (DYX1C1), has been shown to be associated with deficits in short-term memory in dyslexic populations. The purpose of the current study was to examine the behavioral and neuroanatomical phenotype of a mouse model with a homozygous conditional (forebrain) knockout of the rodent homolog Dyx1c1. Twelve Dyx1c1 conditional homozygous knockouts, 7 Dyx1c1 conditional heterozygous knockouts and 6 wild-type controls were behaviorally assessed using auditory discrimination, motor, and learning/memory tasks. Mice with the homozygous Dyx1c1 knockout showed deficits on radial-arm and T-maze tasks, but not on auditory or motor tasks. Following behavioral assessment, all brains were extracted and used for histological evaluation. Volume measures of the striatum (caudate putamen, nucleus accumbens), cortex, and hippocampus were recorded and analyzed. These neuroanatomical findings were correlated with behavioral measures to evaluate whether regional anomalies might account for the learning deficits observed in the Dyx1c1 conditional homozygous knockouts. Our findings affirm existing evidence that DYX1C1 may play an underlying role in the development of neural systems important to learning and memory, and disruption of this gene's function could contribute to learning deficits as seen in individuals with dyslexia.

Disclosures: A.R. Rendall: None. **A. Tarkar:** None. **H.M. Contreras-Mora:** None. **J. LoTurco:** None. **R.H. Fitch:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.03/G25

Topic: C.06. Developmental Disorders

Support: Lee Pesky Learning Center

Title: Statistical learning of spatial cues in virtual hebb-williams maze

Authors: L. GUO[1], Y. CHEN[2], *A. BATTISON[3], L. GABEL[4];
[1]Psychology, [2]Computer Sci., [4]Psychology & Neurosci., [3]Lafayette Col., Easton, PA

Abstract: Statistical learning has been shown in different domains of cognitive abilities, including social understanding, logical reasoning, and language acquisition. The current study explored statistical learning in navigation. Navigators often use spatial cues to support their performance. We proposed that cues associated with high reliability would be adopted more readily relative to unreliable cues. The present study investigated the effect of cue reliability on adults' performance in virtual reality mazes. Undergraduate students performed a navigational task in a virtual Hebb-Williams maze. Participants were randomly assigned with the same sets of mazes that either had no cue, a cue (i.e. a checkerboard pattern implemented on a maze wall) that was reliable, or the same cue less reliable in identifying the correct path to a goal. We measured individual performance efficiency as an indicator of the person's maze performance. Our results supported our major prediction such that adults exposed to the reliable cue outperformed those who had no cue and those with the unreliable cue. The current study should provide useful knowledge for future studies of statistical learning in navigational tasks.

Disclosures: L. Guo: None. **Y. Chen:** None. **A. Battison:** None. **L. Gabel:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.04/G26

Topic: C.06. Developmental Disorders

Support: R01 DA-15403

R01 DA-026485

Title: Sex-dependent effects of adolescent methylphenidate and guanfacine on impulsive choice and cocaine self-administration

Authors: K. J. NORMAN[1], *J. L. LUKKES[2], B. S. THOMPSON[1], S. L. ANDERSEN[3];
[1]Lab. of Developmental Neuropharm., McLean Hosp., Belmont, MA; [2]Lab. of Developmental Neuropharm., McLean Hospital, Harvard Med. Sch., Belmont, MA; [3]Lab. of Developmental Neuropharm., McLean Hospital/Harvard Med. Sch., Belmont, MA

Abstract: Sex-dependent effects of adolescent methylphenidate and guanfacine on impulsive choice and cocaine self-administration **K. J. NORMAN**, J. L. LUKKES, B. S. THOMPSON, and S.L. ANDERSEN Laboratory of Developmental Neuropharmacology, Dept. of Psychiatry, McLean Hospital/Harvard Medical School, Belmont, MA 02478, USA. Methylphenidate (MPH; a dopamine transporter inhibitor) and guanfacine (GUAN; an α_{2a} noradrenergic receptor agonist) treatments effectively increase attentiveness and decrease impulsivity in clinical populations of adolescent ADHD. However, their long-term effects on different aspects of drug addiction, such as motivation to use drugs or sensitivity shifts, have not been thoroughly characterized. The current studies investigate the effect of juvenile to adolescent exposure to MPH or GUAN on concurrent impulsivity and later cocaine self-administration in adulthood. Male and female Sprague-Dawley rats were given MPH (2 mg/kg, p.o.) or GUAN (0.2mg/kg, i.p.) daily beginning on postnatal day (P) 22 and throughout adolescence. Twenty minutes following treatment, rats were placed into an operant conditioning chamber and impulsive choice was assessed using a delay discounting task (DDT; 0, 10, 20, 40, and 60 sec delay) with food reward. Beginning in adulthood (P90), rats were implanted with a jugular catheter and began cocaine self-administration on a fixed-ratio (FR1 and 5, 0.5 mg/kg) and a progressive ratio (PR, 0.25 mg/kg and 0.75 mg/kg) schedule, followed by an FR5 dose-response (0, 0.03, 0.1, 0.3, and 1 mg/kg) assessment. During DDT, MPH increased the number of large food rewards received at the 20 and 40 sec delay in males, suggesting decreased impulsivity, while both MPH and GUAN increased the number of large rewards received at the 20 sec delay in females. In adulthood, MPH and GUAN decreased cocaine intake in males, but had no effect in females. PR breakpoint was reduced by MPH and GUAN in males, but only MPH was effective in females. A downward shift in FR5 responding at 0.03mg/kg in MPH and GUAN treated male and female rats indicates decreased sensitivity to cocaine. These findings suggest that sex-dependent neural adaptations following dopaminergic and noradrenergic activation during development reduce impulsive choice and later sensitivity to and suppression of cocaine intake during adulthood. The project described was supported by R01 DA-15403 and R01 DA-026485 to SLA.

Disclosures: K.J. Norman: None. **J.L. Lukkes:** None. **B.S. Thompson:** None. **S.L. Andersen:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.05/G27

Topic: C.06. Developmental Disorders

Support: start-up grant from Michigan State University

Title: Neural correlates of phonological and orthographic processing in children with developmental dyslexia

Authors: *X. YAN[1], Y. DENG[2], F. CAO[1];
[1]Communicative Sci. and Disorders, Michigan State Univ., East Lansing, MI; [2]Inst. of Psychology, Chinese Acad. Sci., Beijing, China

Abstract: Introduction: Converging behavioral and neurological evidence from alphabetic languages has demonstrated phonological deficits in children with developmental dyslexia (DD) (Bruck, 1992; Rack, Snowling, & Olson, 1992)). However, whether phonological deficit is also the core problem in children with DD in logographic languages is still a debate (Siok & Fletcher, 2001; Siok, et al., 2004). Our study provides neurological evidence for whether Chinese children with DD also experience phonological deficits in an auditory rhyming task using fMRI. Reading is a complex process and skilled orthographic processing also plays an important role in fluent reading (Siok et al., 2004; Cao et al., 2008), especially in logographic languages such as Chinese. Our study also examined whether there are orthographic deficits in Chinese children with DD. **Method:** 5 dyslexic fifth-graders (DD), 13 typically developing fifth-graders (age control, AC) and 14 typically developing third-graders (reading-matched control, RC) participated in an auditory rhyming task and a visual spelling task during fMRI scanning. During the auditory rhyming task, participants judged if two orally presented words rhymed or not; during the visual spelling task, participants judged if the second character of two visual presented words shared a radical or not. **Result & Discussion**: Behavior results showed no significant differences in reaction time (RT) or accuracy among the three groups in the visual spelling task. For the auditory rhyming task, the AC group was significantly faster and more accurate than the DD group and the RC group. fMRI results indicated that in both tasks AC showed greater activation than DD in two IFG regions: the left dorsal IFG (-48, 8, 34) and the left anterior IFG (-50, 34, 18). These two regions have been found to show developmental increase in our previous studies using the same tasks (Cao et al, 2011, 2012). It suggests that children with DD have a developmental delay in these two regions. We also found DD showed reduced activations in comparison to both AC and RC in left middle occipital gyrus (MOG) for the auditory rhyming task and in left cuneus for the visual spelling task. The reduced activation in MOG suggests a weaker integration of orthography and phonology during the rhyming judgment (Fiebach et al, 2002; Gold et al, 2007); the reduced activation in ceneus suggests less elaborated visual-spatial processing of Chinese words (Kim et al, 2015). Both of these mechanisms are related with the etiology of DD rather than the result of being DD.

Disclosures: X. Yan: None. **Y. Deng:** None. **F. Cao:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.06/G28

Topic: C.06. Developmental Disorders

Support: Korea Healthcare Technology Research and Development Project, Ministry for Health and Welfare Affairs (A120013)

Loma Linda University

Title: Shared prefrontal cortical gene expression profiles between adolescent SHR/NCrl and WKY/NCrl rats showing attentional deficits

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Abstract: Factor analyses of attention-deficit/hyperactivity (ADHD) symptoms divide the behavioral symptoms of ADHD into two separate domains, one reflecting inattention and the other, a combination of hyperactivity and impulsivity. Identifying domain-specific genetic risk variants may aid in the discovery of specific biological risk factors for ADHD. In contrast with data available on genes involved in hyperactivity and impulsivity, there is limited information on the genetic influences of inattention. Transcriptional profiling analysis in animal models of disorders may provide an important tool to identify genetic involvement in behavioral phenotypes. To explore some of the potential genetic underpinnings of ADHD inattention, we examined common differentially expressed genes (DEGs) in the prefrontal cortex of SHR/NCrl, the most validated animal model of ADHD and WKY/NCrl, animal model of ADHD-inattentive type. In contrast with Wistar rats, strain representing the “normal” heterogeneous population, SHR/NCrl and WKY/NCrl showed inattention behavior in the Y-maze task. The common DEGs in the PFC of SHR/NCrl and WKY/NCrl vs. Wistar rats are those involved in transcription, synaptic transmission, neurological system process, and immune response. qRT-PCR analyses validated expression patterns of genes representing the major functional gene families among the DEGs. Although further studies are warranted, the present findings indicate novel genes associated with known functional pathways of relevance to ADHD which are assumed to play important roles in the etiology of ADHD-inattentive subtype.

Disclosures: I. dela Pena: None. **J. de la Pena:** None. **B. Kim:** None. **D. Han:** None. **J. Cheong:** None.

Poster

683. Developmental Disorders: Other

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The Grainger Foundation

Title: Thalamic centromedian/parafascicular complex DBS in Tourette syndrome modulates motor and non-motor networks

Authors: *P. TESTINI, W. S. GIBSON, H.-K. MIN, K. R. GORNY, J. P. FELMLEE, K. M. WELKER, C. A. EDWARDS, M. L. SETTELL, E. K. ROSS, B. T. KLASSEN, K. H. LEE; Mayo Clin., Rochester, MN

Abstract: Introduction: Deep brain stimulation (DBS) of the thalamic centromedian/parafascicular complex (CM/Pf) is a therapeutic option for severe, medication-refractory Tourette syndrome (TS). The neural networks that mediate the therapeutic effect have yet to be identified. We performed fMRI in human patients undergoing thalamic DBS for TS to test the hypothesis that DBS of CM and Pf would modulate sensorimotor and limbic networks. Methods: Five patients underwent bilateral stereotactic implantation of DBS electrodes (Medtronic 3387) in the CM/Pf. Electrode location was verified by postoperative CT. Intraoperative 1.5T functional MRI (fMRI) was acquired during DBS of the CM (0-1+ 3V 130Hz 90us) and Pf (2-3+ 3V 130Hz 90us) nuclei. All pulse sequences were previously safety tested in a phantom. At three months post-surgery, a double-blinded tic evaluation using the Rush Video-Based Tic Rating Scale was performed during sham, CM, and Pf stimulation, and these scores were correlated with changes in blood oxygen level-dependent (BOLD) activity. All procedures were approved by the Mayo Clinic Institutional Review Board. Results: Group analysis ($pBonf < 0.001$) showed that CM DBS resulted in an increase in BOLD signal within motor regions including the ipsilateral putamen, pallidum, motor and premotor cortices, thalamus, and contralateral cerebellum. Stimulation of Pf, however, resulted in a decrease in BOLD signal within these same motor regions, as well as in limbic and associative areas including the insula and cingulate cortices. Both CM and Pf DBS caused clinically relevant improvements in Rush scores relative to sham stimulation ($p = 0.12, 0.03$; paired t-test). A positive linear correlation between DBS-evoked improvement in Rush scores and magnitude of BOLD signal change was observed in the ipsilateral motor cortex and contralateral cerebellum.

Conclusions: These results show that CM and Pf DBS exert differential effects on sensorimotor and limbic networks. Notably, the observed effect of both CM and Pf DBS on the motor network BOLD activation, and the correlation between such activation and the clinical outcomes suggest that DBS-evoked modulation of the motor circuitry may play a key role in the therapeutic mechanism of CM/Pf DBS. Acknowledgements: This work was supported by the National Institutes of Health (R01 NS 70872 awarded to KHL) and by The Grainger Foundation.

Disclosures: P. Testini: None. **W.S. Gibson:** None. **H. Min:** None. **K.R. Gorny:** None. **J.P. Felmlee:** None. **K.M. Welker:** None. **C.A. Edwards:** None. **M.L. Settell:** None. **E.K. Ross:** None. **B.T. Klassen:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic, Boston Scientific. **K.H. Lee:** None.

Poster

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Title: Disrupted white matter connectivity in dyslexic children: a multivariate pattern analysis

Authors: *Z. CUI, Z. XIA, H. SHU, G. GONG;
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Abstract: Disrupted brain connectivity between reading related regions has been hypothesized to be a critical contributing factor for developmental dyslexia (DD). The existing white matter (WM) studies in dyslexia always treated voxels or tracts as independent variables and have difficulties in detecting subtle and spatially distributed differences. In the present study, we apply a multivariate pattern analysis to test if the DD can be characterized by atypical distributed WM patterns. Twenty-eight dyslexic children and 33 matched typically developing controls were included. For each individual, the mean of WM volume (WMV), fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) for each White Matter Parcellation Map region were computed and then concatenated into a single feature vector. Then, a linear support vector machine (LSVM) classifier was constructed to discriminate dyslexics and controls (Figure 1). To validate our results, the logistic regression (LR) model was applied to classify the dyslexics and controls with the exact same classification framework. The prediction accuracy of the proposed LSVM classifier was 83.61% (P<0.001; sensitivity=75.00% and specificity=90.91%). The AUC was 0.86 (P<0.001). There are 43 discriminative WM features, which were mainly located within the putative reading system (e.g., the superior longitudinal fasciculus), the limbic system (e.g., the cingulum and fornix), and the motor control system (e.g., the inferior cerebellar peduncle). The prediction accuracy and AUC of the LR classifier were 73.77% and 0.80 (all P<0.001). The weights of the discriminative features for the LR classifier were significantly correlated with that for the LSVM classifier (r=0.73, P<0.001). These results demonstrated a significant WM pattern change in dyslexia, which were mainly associated with WM tracts in the putative reading network, limbic system and motor system, and further highlight the potential of WM connectivity as a biomarker for recognizing brain disorders or diseases.

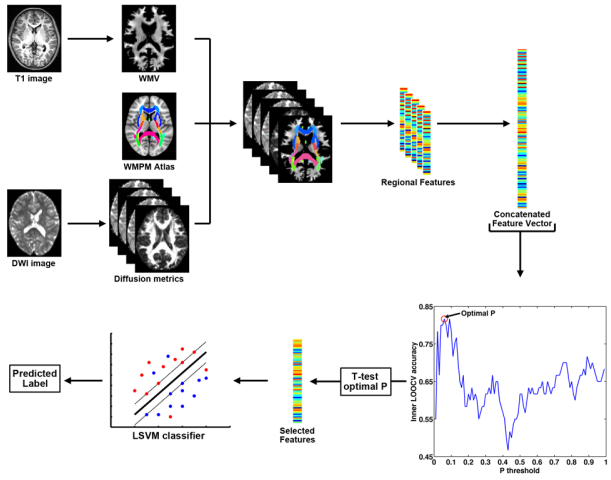

Figure 1. The classification flow based on the combination of white matter volume and diffusion metrics. Two-tailed two sample t-test was used for feature selection and nested LOOCVs were applied for the optimal P threshold.

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Poster

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Support: National Initiative Brain and Cognition (NIHC) Grant 056-14-015

Title: Reduced electrophysiological connectivity during visual word recognition in dyslexic children

Authors: *G. ŽARIC[1], J. M. CORREIA[1], G. FRAGA GONZÁLEZ[2], J. TIJMS[3], M. W. VAN DER MOLEN[2], L. BLOMERT[1], M. BONTE[1];
[1]Cognitive Neurosci., Maastricht Univ., Maastricht, Netherlands; [2]Dept. of Developmental Psychology, Univ. of Amsterdam, Amsterdam, Netherlands; [3]IWAL institute, Amsterdam, Netherlands

Abstract: Reading is a complex cognitive skill subserved by a distributed network of visual and language-related regions. Disruptions of connections within this network are proposed as a possible cause of reading dysfunction in developmental dyslexia. Here we investigated effective connectivity in the reading network of 9-year-old typically reading children (TR; n=20) and two groups of dyslexic children: severely dysfluent dyslexic (SDD; n=20) and moderately dysfluent dyslexic (MDD; n=18). To this end, we used directed transfer function (DTF) to analyze the electroencephalographic (EEG) signal recorded while the children recognized visual words and meaningless letter-like symbol strings. DTF is a spectral multivariate estimator of EEG activity propagation and based on autoregressive models. Here DTF was performed per subject and random-effect statistics were calculated between groups per condition using Wilcoxon tests per frequency bin (FDR<0.05). Our preliminary analysis indicates an expected propagation from posterior to anterior channels in TR children during both the visual word and symbol string recognition task. Similar results were found in the MDD group with significantly weaker connectivity from left parietal to central and frontal channels than in TRs on both tasks. On the other hand, the SDD readers showed significantly weaker propagation from occipital to parietal and central channels on both tasks than both TR and MDD groups. Most interestingly, the SDD group exhibited significantly stronger bilateral anterior to posterior connectivity in both conditions than TR and MDD groups, spanning mostly in the lower gamma, but also alpha and beta bands. This pattern of results suggests that the two groups of dyslexic children use two different compensatory mechanisms: MDD readers rely on qualitatively the same connectivity as TRs; while SDD readers more strongly rely on bilateral anterior to posterior connectivity. This differentiation emphasizes the importance of considering differences in severity of reading

dysfunction within dyslexic population in addition to group differences between typical and dyslexic readers.

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Poster

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Support: FP7/2007-2013 TACTICS 278948

NWO VENI 016.135.023

Title: Gut microbiome in ADHD and its relation to brain function

Authors: ***E. AARTS**[1], T. EDERVEEN[2], J. NAAIJEN[3], M. ZWIERS[1], J. BOEKHORST[4], H. TIMMERMAN[4], J. GLENNON[3], B. FRANKE[3], R. COOLS[3], J. BUITELAAR[3], S. VAN HIJUM[2,4,5], A. ARIAS VASQUEZ[3];
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Abstract: Human commensal microbial communities in the intestine (i.e. the gut microbiome) have an increasingly recognized impact on human health, including brain functioning. Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by abnormal brain dopamine; for instance reflected by decreased reward anticipation responses in the ventral striatum in the brain. Of the many etiological factors involved in ADHD, the microbiome might constitute an important one. Here, we investigated the difference in microbiome between ADHD cases and undiagnosed controls, as well as its effects on brain functioning. Using next-generation 454 DNA sequencing of the bacterial 16s rRNA marker gene, we characterized microbial communities of an n = 97 ADHD cases and controls cohort. Firstly, we investigated the differences in abundance of (i) bacterial taxa and (ii) predicted metabolic functions of the microbiome based on the presence and abundance of bacterial taxa. Secondly, we assessed neural reward anticipation responses using fMRI in an n = 87 ADHD cases and

controls cohort. Thirdly, in a sub-set of 28 subjects who participated in both the microbiome and fMRI study, we related differences in microbiome to neural reward anticipation. Many bacterial taxa differed between cases and controls, among which the Bifidobacterium genus that was increased from 12.0% to 20.2% in average relative abundance in ADHD cases ($p = 0.002$, MWU). When we focused our analyses on genes producing monoamine (including dopamine) precursors in the bacteria, we found that a gene involved in the synthesis of the essential amino acid phenylalanine ((KEGG K01713) encoding the enzyme prephenate dehydratase (EC: 4.2.1.51)) was significantly more present in the microbiome of ADHD cases compared with controls. In our larger imaging sample, reward anticipation in the anatomically defined ventral striatum was significantly decreased for ADHD cases relative to controls, replicating previous studies. Strikingly, in our smaller sub-sample, increased abundance of this microbial phenylalanine-related gene was also associated with decreased bilateral ventral striatal BOLD responses for reward anticipation ($pFWE < 0.05$, small search volume: anatomical ventral striatum), independent of ADHD diagnosis. Our results suggest that differences in gut microbiome structure exist between ADHD cases and controls. Importantly, relative abundance of a dopamine-related microbial gene that differed between ADHD cases and controls was associated with altered reward anticipation responses, one of the neural hallmarks of ADHD.

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Poster

683. Developmental Disorders: Other

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The Escher Fund for Autism.

Title: A father’s exposure to nicotine and its surprising consequences for the offspring

Authors: *D. M. MC CARTHY, S. E. LOWE, T. J. MORGAN, Jr, E. N. CANNON, F. FAN, J. ZHU, P. G. BHIDE;
Ctr. for Brain Repair, Biomed. Sci., Florida State University,College of Med., Tallahassee, FL

Abstract: Much attention has been focused on the consequences of tobacco use by pregnant women or women of childbearing age and its effects on the developing fetus. A number of clinical and pre-clinical studies show a relationship between fetal nicotine exposure and premature birth, sudden infant death syndrome, and an increased risk of attention deficit hyperactivity disorder (ADHD). However, in reality, smoking is more prevalent among men (21.6%) than women [(16.5%) (Centers for Disease Control)]. Whether a father's use of tobacco affects cognitive development of the offspring he sires is not clear. To address this issue, we developed a mouse model of paternal nicotine exposure in which adult male mice were exposed to nicotine in drinking water for up to 12 weeks and then bred with drug naïve female mice. The nicotine was dissolved in saccharin sweetened drinking water to mask nicotine's bitter taste. Thus, we created three experimental groups of mice exposed either to plain drinking water, 2% saccharin or nicotine (200 μg/ml) plus 2% saccharin. Since our goal was to determine if paternal exposure to nicotine is associated with ADHD phenotypes in the offspring, we analyzed working memory, attention and spontaneous locomotor activity in the offspring sired by males in each treatment group. Hyperactivity, working memory deficit and attention deficit are major components of the ADHD symptomatology. The offspring of the nicotine exposed males showed significant deficits in working memory and attention but not in locomotor activity. The offspring of the saccharin exposed males showed significant increase in locomotor activity, and did not show significant changes in working memory or attention. Thus, paternal exposure to nicotine or saccharin each produced distinct behavioral consequences for the offspring that are associated with the ADHD phenotype.

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Poster

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Doctoral Programme of Psychology, Learning and Communication (PsyCo)

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Title: Distinct associations between neural speech discrimination responses and verbal cognition in dyslexic and fluently reading children

Authors: *L. KIMPPA[1], E. PARTANEN[3], K. ALHO[4], T. KUJALA[2];
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Abstract: The relationship between the underlying neural deficit and difficulties in cognitive skills in dyslexia is ill-defined. We explored the association of neural indices of auditory discrimination and behavioural-level language and verbal memory functions in dyslexic and fluently reading 9-12-year-old children. Responses to changes in frequency, vowel identity and duration either at the middle or final syllable of a tri-syllabic pseudo-word were recorded in an oddball paradigm. Mismatch negativity (MMN), P3a and late discriminative negativity (LDN) event-related brain potentials (ERPs) were measured in an ignore condition where children focussed on watching a silent cartoon. Correspondingly, N2, P3a and LDN were measured in an attend condition where the children were instructed to attend to the speech stimuli and press a button to a novel target sound. Cognitive skills were tested on several domains: conceptual/abstract verbal reasoning and phonological processing, rapid automatized naming (RAN), verbal working memory, verbal learning and long-term memory, as well as reading fluency and writing. The association between response amplitudes and cognitive performance was assessed using stepwise multiple regression analysis. Group differences were investigated by conducting multiple regression analysis separately for each group and response. In the dyslexics, several connections between early neural discrimination of sounds, indexed by the MMN and N2, and especially RAN time were found, whereas the controls' early responses were linked to verbal reasoning and working memory. The attention-related P3a responses were associated with verbal reasoning and learning in the dyslexics and rapid naming time and working memory in the controls. The LDN, reflecting late discriminative ability and/or reorienting, was related to error-proneness and auditory short-term memory functions in dyslexics; in controls it was most strongly related to verbal learning and later memory retrieval. The only significant relationship with reading fluency was found for duration N2 in dyslexics. In sum, the MMN, N2 and P3a responses predicted verbal working memory performance while the LDN predicted long-term memory performance in the controls but no such pattern was observed in the dyslexics. Significant relationships between the neural responses and measures of language and memory functions were found in both groups, but the couplings differed. These results imply somewhat distinct connections of low-level speech processing and higher-level verbal cognition in dyslexia compared to fluently reading individuals.

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Poster

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Support: R03 HD048752

R15 HD065627

Title: The basal ganglia in relation to executive dysfunction in ADHD and reading disorder

Authors: ***A. C. JAGGER**, A. A. OLECHOWSKI, L. ALVES, M. Y. KIBBY;
Psychology, Southern Illinois Univ. - Carbondale, Carbondale, IL

Abstract: The basal ganglia (BG) has strong connections to frontal cortex. There are frontostriatal circuits for motor functioning, executive functioning (EF), and motivation (Tekin & Cummings, 2002). We recently found BG gray matter volume is smaller in children with ADHD, reading disability (RD) and comorbid ADHD and RD (RD/ADHD) compared to controls (Jagger et al., 2015). The frontostriatal network is often atypical in ADHD, particularly in the prefrontal cortex and caudate (Shaw et al., 2014; Cortese et al, 2005) and in white matter tracts (Silk et al., 2009). Individuals with RD show reduced BG function in relation to higher order learning (Howard et al, 2006). Our study examined whether BG gray matter volume may be differentially related to cognitive and behavioral EF measures in RD, ADHD, and RD/ADHD. Over 100 children (30 controls, 22 RD, 15 RD/ADHD and 42 ADHD; 55 males) participated in the study, ranging in age from 8-12 years. T1 weighted MRI scans were collected on all participants and analyzed using voxel-based morphometry (VBM) in SPM 8 with a BG mask. EF was assessed with the Behavior Rating Inventory of Executive Function (BRIEF) questionnaire completed by the parents on the child. A t-test was used to validate the volume difference between controls and the clinical groups. Multiple regressions were then conducted within group in VBM, between BG gray matter volume and cognitive EF measures (BRIEF scores of planning/organizing (P/O) and working memory (WM) and behavioral EF measures (BRIEF scores of emotional control and behavioral regulation) to observe whether there is a relationship between EF and BG volume. The BG was smaller in the clinical groups compared to the control group. For children with ADHD, smaller caudate volume bilaterally was related to worse P/O, and smaller right lenticular nucleus and left globus pallidus volumes were related to poor WM.

For the RD group, smaller right putamen volume was related to worse P/O. In the comorbid group, smaller left putamen and bilateral caudate volume was related to poor WM. In addition, smaller left caudate volume was related to worse P/O. Our results strengthen prior conclusions that alterations in the BG in ADHD are associated with deficits in EF. Based on our findings, these relations are specific to cognitive EF, contributing to current notions of the functions of the dorsolateral prefrontal-striatal circuit. In RD this relation is specific to higher order processing (P/O). Those with comorbid RD/ADHD generally have relationships between BG volume and cognitive EF consistent with having ADHD; however, the hemisphere and specific nucleus involved varied somewhat. Network analysis would be a beneficial next step.

Disclosures: **A.C. Jagger:** None. **A.A. Olechowski:** None. **L. Alves:** None. **M.Y. Kibby:** None.

Poster

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Topic: C.06. Developmental Disorders

Support: NIH grant R21-MH080820

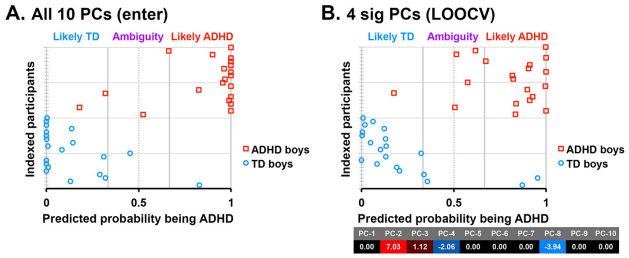
NIH training grant T32-NS047987

Title: Functional neuroimaging of visuospatial working memory tasks enables accurate identification of attention deficit and hyperactivity disorder

Authors: *R. HAMMER[1,2], G. E. COOKE[3], M. A. STEIN[4], J. R. BOOTH[5]; [2]Interdepartmental Neurosci. Program, [1]Northwestern Univ., Evanston, IL; [3]Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois, Urbana-Champaign, Urbana-Champaign, IL; [4]Dept. of Psychiatry and Behavioral Sci., Univ. of Washington Sch. of Med., Seattle, WA; [5]Dept. of Communication Sci. and Disorders, The Univ. of Texas at Austin, Austin, TX

Abstract: Finding neurobiological markers for neurodevelopmental disorders, such as Attention Deficit and Hyperactivity Disorder (ADHD), is a major objective of clinicians and neuroscientists. Here we examined if functional Magnetic Resonance Imaging (fMRI) data from few distinct visuospatial working memory (VSWM) tasks allows accurate identification of cases with ADHD. We tested 20 boys with ADHD combined type and 20 typically developed (TD) boys in four VSWM tasks that differed in feedback availability (no-feedback, feedback) and

reward size (large, small). We used a multimodal analysis based on brain activity in 16 regions of interest, significantly activated or deactivated in the four VSWM tasks (based on the entire participants' sample). Dimensionality of the data was reduced into 10 principal components that were used as the input variables to a logistic regression classifier. We found that fMRI data from the four VSWM tasks enabled classification accuracy of 92.5%, with high predicted ADHD probabilities values for most clinical cases, and low predicted ADHD probabilities for most TDs. This accuracy level was higher than those achieved by using the fMRI data of any single task, or the respective behavioral data. These findings indicate that task-based fMRI data acquired while participants perform few distinct VSWM tasks enables better identification of clinical cases. Our findings also provide an important theoretical contribution, showing that the manifestation of neurocognitive abnormalities in ADHD is context dependent. We suggest that similar approach can be adopted for diagnosing other clinical populations, and possibly also for dissociating between distinct clinical populations who share behavioral symptoms. This would require using cognitive tasks that target the neurocognitive deficits characterizing the clinical condition of interest, or a battery of tasks that may enable dissociating between distinct neurocognitive abnormalities.



Disclosures: **R. Hammer:** None. **G.E. Cooke:** None. **M.A. Stein:** None. **J.R. Booth:** None.

Poster

683. Developmental Disorders: Other

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Topic: C.06. Developmental Disorders

Title: Variability in the auditory-evoked neural response as a potential mechanism for dyslexia

Authors: ***T. M. CENTANNI**[1], D. PANTAZIS[1], L. DENNA[2], J. D. E. GABRIELI[1], T. P. HOGAN[2];
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Abstract: Recent work has demonstrated that rat models of dyslexia exhibit increased trial-by-trial variability in the primary auditory cortex response to sound and that this feature is also present in the auditory brainstem response of children with dyslexia. Whether this feature is present in cortex and whether this represents a reliable biomarker for this disorder is still unknown. In the current study, we investigated whether trial-by-trial neural variability is present in auditory and/or visual cortex of children with dyslexia using magnetoencephalography (MEG); a non-invasive brain imaging technique with millisecond temporal precision. Recordings were acquired in response to auditory speech sounds and tones as well as visual presentation of letters and objects. Response consistency was calculated in three ways using Pearson's r; (1) individual trial comparisons to test general variability, (2) trials from the beginning of the session compared with trials from the end of the session to evaluate neural fatigue, and (3) even numbered trials versus odd numbered trials to evaluate consistency of the result. Our results demonstrate that decreased consistency in the neural response is present in cortex of children with dyslexia compared to typically developing children. This effect is present not only when all possible repetitions are compared, but also when responses from the beginning of the session are compared with trials at the end of the session. These results support the recent theory that neural variability could be a neural mechanism for dyslexia.

Disclosures: T.M. Centanni: None. **D. Pantazis:** None. **L. Denna:** None. **J.D.E. Gabrieli:** None. **T.P. Hogan:** None.

Poster

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Support: Young Investigator award to LAR

Title: Modeling attention-deficit hyperactivity disorder in the albino rat: comparison between spontaneously hypertensive rats, wistar-kyoto hyperactive, wistar-kyoto hypertensive and naples high excitability lines

Authors: E. CARBONI[1], L. A. RUOCCO[2], C. TRENO[2], U. GIRONI CARNEVALE[2], F. SADILE[3], R. SERPE[4], R. ASPIDE[5], *A. G. SADILE[6];
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Abstract: Modeling neuropsychiatric problems, such as Attention-Deficit Hyperactivity Disorder (ADHD), autism and others has been useful to the understanding of elementary components of behavior. The aim of the present studies was to compare across various models of ADHD available in the literature, two behavioral traits, namely free rearing or leaning against the walls. The model systems used were the Spontaneously Hypertensive (SHR), Wystar-Kyoto Hyperactive (WK-HA), Wystar-Kyoto Hypertensive (WK-HT) and the Naples High Excitability (NHE) line. The experimental design included young adult male rats that were tested in a spatial novelty test, the Làt-maze, for 10 min. Behaviour was videotaped and analyzed for frequency of corner crossing (HA), frequency and duration of rearing (R) and leaning (L). Duration of rearing and leaning operationally define non-selective attention (NSA). Results showed (i) a higher frequency of rearing and leaning in SHR than in Wystar-Kyoto controls, (ii) a higher frequency of rearing and leaning and longer duration of leaning in WK-HA than in WK-HT rats, (iii) a higher frequency and shorter duration of leaning in NHE rats than in Naples Random-bred controls. Moreover, these studies showed a higher frequency of rearing and leaning in hyperactive SHR and WK-HA rats and a higher frequency of shorter duration leaning in NHE. In conclusion, this series of studies on rearing and leaning in genetic model systems revealed that these are dissociable traits. This, in turn, may help to understand the neural mechanisms of cognitive and non-cognitive aspects of behavior.

Disclosures: E. Carboni: None. **L.A. Ruocco:** None. **C. Treno:** None. **U. Gironi Carnevale:** None. **F. Sadile:** None. **R. Serpe:** None. **R. Aspide:** None. **A.G. Sadile:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.17/G39

Topic: C.06. Developmental Disorders

Support: Lee Pesky Learning Center

Title: Virtual hebb williams maze: a practicable early detection method for dyslexia?

Authors: L. GUO[1], N. ESCALONA[2], Y. CHEN[2], R. SZTEINBERG[2], D. TRUONG[5], A. BATTISON[3], J. BOSSON-HEENAN[5], J. PFAFFMANN[2], J. R. GRUEN[6], E. JOHNSON[7], *L. A. GABEL[4];

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Abstract: Dyslexia, or Reading Disability (RD), is a specific impairment in processing written language despite adequate intelligence and educational background. RD, affecting 5-17% of school-aged children, has far-reaching social and economic consequences. Genetic studies have identified multiple candidate dyslexia susceptibility genes (CDGSs). Multiple theories of RD have emerged with the majority of available data pointing to phonological and visual deficits. Genetically altering CDSG expression in mice results in similar visual and auditory processing deficits to those identified in humans. Despite the similarities, it remains unclear whether reported deficits in animal models directly translate to RD in humans. Recent studies have demonstrated that humans using a virtual representation of a Hebb-Williams (vHW) maze task (measure of visual-spatial processing) perform similarly to mice using the standard apparatus in the laboratory. Data from our laboratory demonstrate that adults (18-22 years old) and children (8-13 years old) exhibit similar performance efficiencies on a vHW to mice completing the physical HW maze. These data suggest that humans share common problem solving skills on our vHW maze with mice completing the physical version of the maze. In the current study we examined performance of children (5-6 years of age, and 8-13 years of age) with and without RD on the vHW maze. Children with RD, in both age groups, exhibited impaired performance on this task, similar to animal models of RD. Path analysis results show commonalities in problem solving ability in this impaired group, which may lead to a marker specific to individuals with RD. Preliminary data suggest that typically developing adults (18-23 years of age) use statistical learning strategies to successfully navigate the vHW maze, but it is still unclear what strategies children with RD employ to complete the task. These data, combined with genetic analysis of CDSG expression, will provide powerful information toward our long-term goal of early identification of children at risk for reading disorder.

Disclosures: L. Guo: None. **N. Escalona:** None. **Y. Chen:** None. **R. Szteinberg:** None. **D. Truong:** None. **A. Battison:** None. **J. Bosson-Heenan:** None. **J. Pfaffmann:** None. **J.R. Gruen:** None. **E. Johnson:** None. **L.A. Gabel:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.18/G40

Topic: C.06. Developmental Disorders

Title: Animal model of cognitive dysfunction responding to ADHD therapies

Authors: E. ANDRIAMBELOSON[1], E. POIRAUD[1], B. HUYARD[1], L. GORJ[1], *S. M. O'CONNOR[2], S. WAGNER[1];
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Abstract: Over 90% of adults and children living with and seeking treatment for Attention Deficit Hyperactivity Disorder (ADHD) manifest cognitive dysfunction, particularly impairments in attention, working memory and executive function which provides support for a cognitive rather than psychomotor basis of ADHD pathology. The existing animal models for ADHD feature psychomotor behavior impairments (impulsivity and hyperactivity) but do not always favorably respond to the psychostimulant drugs used for the treatment of ADHD. In the present study, the potential of ADHD médications (methylphenidate, amphetamine and atomoxetine) to restore cognitive performance (spontaneous and continuous alternation in the T-maze) was tested in mice after pharmacological alteration of the central cholinergic system by injection of scopolamine. Amphetamine, Methylphenidate and Atomoxetine elicited dose-dependent reversion of cognitive deficit in scopolamine -treated mice. Seven days of subchronic treatment was required to obtain the cognitive effect of amphetamine whereas Methylphenidate and Atomoxetine were effective following a single acute or 3-day subchronic dosing regimen. Furthermore, Atomoxetine was effective after a short pretreatment period (0.5h prior to the T-maze task) whereas Amphetamine and Methylphenidate required longer pretreatment times (16h). The above results demonstrate that psychostimulant drugs with differing mechanisms of action have a positive effect in this model of scopolamine-induced cognitive dysfunction, and indicate its utility for evaluating the cognitive enhancing properties of new chemical entities for ADHD.

Disclosures: E. Andriambeloson: None. **E. Poiraud:** None. **B. Huyard:** None. **L. Gorj:** None. **S.M. O'Connor:** None. **S. Wagner:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: The Jim and Betty Ann Rodgers Chair Funds

Florida State University Grants in Assistance Program.

Title: Prenatal nicotine exposure produces attention deficits in male and female mice

Authors: F. FAN[1], A. GANNON[1], O. N. JACKSON[1], P. S. BOHLEM[1], S. B. STERLING[1], D. MCCATHY[1], T. SPENCER[2], J. BIEDERMAN[2], P. G. BHIDE[1], *J. ZHU[1];
[1]Ctr. for Brain Repair, Biomed. Sci., Florida State Univ. Col. of Med., Tallahassee, FL; [2]Clin. and Res. Programs in Pediatric Psychopharmacology and Adult ADHD, Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: Inattention is a major cognitive symptom of attention deficit/hyperactivity disorder (ADHD). Maternal smoking during pregnancy is linked with a significant increase of risk for ADHD in the exposed offspring. Consistent with this, rodent models of prenatal nicotine exposure (PNE) show a number of deficits associated with ADHD. Here we characterized attention performance in a PNE mouse model using an Object-Based Attention test in which mice are exposed to 5 different objects during the training session and to 2 objects, one novel and one familiar, during the test session. Mice appropriately divide attention (time spent exploring an object) among the five objects in the training session, and then in the test session, focus attention preferentially upon the novel object. A mouse with attention deficit is not expected to favor the novel object, but focus equal attention upon familiar and novel objects. We found that male and female mice in the control group (not prenatally exposed to nicotine) spent significantly longer time exploring the novel object during the test session. However male and female mice in the PNE group spent approximately equal length of time exploring novel and familiar objects. Since methylphenidate (MPH) is among the most commonly used stimulant medications in the treatment of ADHD, we examined the effects of MPH on attention in our PNE model. We found that a single oral dose of MPH (0.75 mg/kg) improved attention in male and female mice in the PNE group such that there was no longer a difference between the PNE and control groups in this measurement. Moreover, similar administration of MPH to the control groups did not affect attention in male or female mice, nor did saline administration to male or female mice in the PNE group produce any effects on attention. Our data show that PNE produces attention deficits in both male and female mice and that MPH improves attention in both sexes. Thus, our data further support the value of the PNE mouse model as an animal model of ADHD.

Disclosures: F. Fan: None. **A. Gannon:** None. **O.N. Jackson:** None. **P.S. Bohlem:** None. **S.B. Sterling:** None. **D. McCathy:** None. **T. Spencer:** None. **J. Biederman:** None. **P.G. Bhide:** None. **J. Zhu:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.20/G42

Topic: C.06. Developmental Disorders

Support: European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 602450 (IMAGEMEND)

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Title: Pattern recognition in stop-signal fMRI classifying ADHD from siblings and controls

Authors: *T. WOLFERS[1,2], D. VAN ROOIJ[1,6], A. MARQUAND[1,7], C. BECKMANN[1,3,8], B. FRANKE[2,4], J. K. BUITELAAR[3,5];
[1]Donders Ctr. for Cognitive Neuroimaging, Radboud Univ., Nijmegen, Netherlands; [2]Dept. of Human Genet., [3]Dept. of Cognitive Neurosci., [4]Dept. of Psychiatry, [5]Karakter Child and Adolescent Psychiatry, Radboud Univ. Med. Ctr., Nijmegen, Netherlands; [6]Dept. of Psychiatry, Univ. Med. Ctr. Groningen, Groningen, Netherlands; [7]Inst. of Psychiatry, King's Col., London, United Kingdom; [8]Ctr. for Functional MRI of the Brain (FMRIB), Univ. of Oxford, Oxford, United Kingdom

Abstract: In earlier studies using pattern recognition, attention deficit hyperactivity disorder (ADHD) could be distinguished from healthy controls on the basis of magnet resonance imaging (MRI) derived features with promising but mixed results. These studies were preformed in small cohorts that did not include siblings. In the present study we use pattern recognition to identify functional MRI patterns that distinguish ADHD from unaffected siblings and healthy controls in a large clinical sample. In total 228 participants, belonging to ADHD-, siblings- or control-group, were tested. The groups were balanced and well matched on age and gender. Each participant preformed a stop-signal task adapted for MRI scanning. Beta weights for the main univariate task contrasts were calculated and used as input for a Gaussian process classifier (GPC). GPCs are best described as a distribution over functions. Based on Bayes' rule the posterior distribution of functions which represents the training data is found in an optimal way. This posterior distribution is used to classify new examples according to the rules of probability.

ADHD could reliably be classified from controls (area under the curve (AUC): .68; $p<.05$) as well as from their unaffected siblings (AUC: .8; $p<.05$). Siblings could not significantly be distinguished from controls. Surprisingly, the classification of ADHD from siblings was better than from controls, although siblings share neurobiological alterations related to ADHD. The present results contribute significantly to the investigation of ADHD with pattern recognition, as it is one of the largest studies performed so far and the only one in a cohort including siblings. It presents realistic estimates for the classification of ADHD based on current methodological developments in machine learning and importantly, is the first that shows the potential of pattern recognition for the diagnostics of ADHD from unaffected siblings. The present finding indicates that pattern recognition in stop-signal functional MRI task might hold the potential for the development of biomarkers for ADHD.

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Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.21/G43

Topic: C.06. Developmental Disorders

Support: NIH grant R01MH62873

FP7 TACTICS 278948

Title: The glutamate pathway is associated with hyperactive/impulsive symptoms and autism symptoms in Attention-Deficit/Hyperactivity Disorder

Authors: *J. NAAIJEN[1], J. BRALTEN[1], S. FARAONE[2], J. GLENNON[1], B. FRANKE[1], J. BUITELAAR[1];
[1]Radboud UMC, Nijmegen, Netherlands; [2]State Univ. of New York Upstate Med. Univ., New York, NY

Abstract: Attention/Deficit Hyperactivity Disorder (ADHD) and Autism Spectrum Disorders (ASD) are highly heritable, but finding the contributing genetic risk variants has turned out to be challenging. Many genetic studies have focused on monoaminergic candidate genes. Current

concepts of neurodevelopmental disorders also implicate glutamate, the most abundant excitatory neurotransmitter in the brain. Altered glutamatergic signaling has been implicated in both ASD and ADHD in 1H-MR spectroscopy studies, genome-wide studies of SNPs and CNVs, candidate gene association and linkage studies. As multiple genes with small effects are assumed to play a role in both ADHD and ASD, considering multiple genetic variants within the same analysis can potentially increase the total explained phenotypic variance and thereby boost the power of genetic studies. In the current study we investigated in an ADHD case-only design, whether glutamatergic signalling pathways are candidate pathways for both ADHD and ASD, and would show association with ADHD symptoms and/or autism symptoms. Phenotypes included ADHD symptom severity (inattention and hyperactivity/impulsivity symptoms) measured with Conners' Parent Scales and autism symptoms measured with the social communication questionnaire (SCQ). Variants within glutamate pathway genes (selected from Ingenuity IPA, www.ingenuity.com) including a 25-kb flanking region were selected for SNP-by-SNP linear regression with our phenotypes of interest, with age and sex as covariate. By summing effects of common genetic variants within the pathway to create an observed summed statistic and comparing this with 10.000 permutations, we were able to compute a pathway-based empirical p-value. This was based on the number of times the summed permuted statistic was more extreme than the summed observed statistic. The selection of genes yielded a total of 66 glutamatergic genes. The pathway analysis for symptoms severity showed a significant association with autism symptoms (Pempircal =.0013) and hyperactivity/impulsivity symptoms (Pempirical = .0010), but not with inattention symptoms (Pempirical = .063). Single gene and single SNP analyses did not show any significant associations. This study is the first to show an association between glutamatergic signalling and autism and hyperactivity/impulsivity symptoms in ADHD using a hypothesis-based pathway association analysis in a case-only design. Further studies investigating a possible link between ADHD and autism based on altered glutamatergic signaling are warranted.

Disclosures: **J. Naaijen:** None. **J. Bralten:** None. **S. Faraone:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Receives royalties from books published by Guilford press and Oxford press. F. Consulting Fees (e.g., advisory boards); Has received consultancy income and/or research support from Akili interactive labs, Alcobra, VAYA pharma and SynapDx. **J. Glennon:** None. **B. Franke:** None. **J. Buitelaar:** F. Consulting Fees (e.g., advisory boards); J. K. Buitelaar has been consultant to/member of advisory boardof and/or speaker for Janssen Cilag BV, Eli Lilly, Bristol-Myer Squibb,Shering Plough, UCB, Shire, Novartis and Servier..

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.22/G44

Topic: C.06. Developmental Disorders

Support: Supported by The Jim and Betty Ann Rodgers Chair Funds and the Escher Fund for Autism.

Title: Methylation of germ cell DNA as the basis for transgenerational transmission of nicotine-induced cognitive phenotypes

Authors: *T. J. MORGAN, JR, D. M. MCCARTHY, S. E. LOWE, P. G. BHIDE;
Biomed. Sci., Florida State Univ., Tallahassee, FL

Abstract: Exposure to environmental chemicals is known to influence our germ cells and therefore future generations. A key area of concern in this regard has been maternal nicotine use and the consequent fetal nicotine exposure. Interestingly, smoking is more prevalent among men (21.6%) than women (16.5%; CDC), and the effects of paternal tobacco use on future generations are not well understood. We have developed a mouse model of paternal nicotine exposure (200 µg nicotine in drinking water for 12 consecutive weeks), in which offspring sired by the nicotine exposed males display significant deficits in cognitive behaviors, particularly working memory and attention (two key ADHD phenotypes). Based on these intriguing findings we hypothesized that the nicotine produces epigenetic changes in the fathers' germ cells and that these changes in the germ cell DNA are the basis for transmission of an ADHD-like phenotype to the offspring. To test this hypothesis, we performed molecular genetic analysis of sperm obtained from nicotine exposed male mice to identify potential nicotine-induced epigenetic modifications of the DNA. Initial findings suggest a 40% decrease in global methylation of the sperm DNA in the nicotine exposed males. To further characterize the epigenetic changes (e.g. methylation or de-methylation) in promoter regions of specific genes, we performed 5-mC immunoprecipitation and subsequent PCR validation of sperm DNA from nicotine-exposed and drug naive males. Parallel *in vitro* studies were conducted to test the influence of direct exposure of the sperm to nicotine and other chemicals. Collectively, our findings establish, for the first time that epigenetic modification of the germ cell DNA is the mechanism of heritability of cognitive and molecular phenotypes produced by paternal nicotine exposure.

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Poster

683. Developmental Disorders: Other

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.23/H1

Topic: C.06. Developmental Disorders

Support: M.FE.A.NEPF0001

Title: Speech-evoked brainstem responses relate to KIAA0319 variants and phonological skills in pre-reading children: a biomarker for dyslexia?

Authors: *E. N. NEEF[1], J. BRAUER[1], A. WILCKE[2], H. KIRSTEN[2], B. MÜLLER[2], M. A. SKEIDE[1], J. LIEBIG[1], I. KRAFT[1], G. SCHAADT[1], N. KRAUS[3], F. EMMRICH[2], A. D. FRIEDERICI[1];
[1]Max Planck Inst., Leipzig, Germany; [2]Fraunhofer Inst. for Cell Therapy and Immunol., Leipzig, Germany; [3]Northwestern Univ., Evanston, IL

Abstract: Developmental dyslexia is a disorder most often accompanied by deficits in phonological awareness, phonemic categorization and speech-in-noise perception. Very early signals of the auditory pathway indicate an abnormal encoding of speech stimuli in reading impaired children. Their speech-evoked brainstem responses are less consistent and distinctive. An insufficient signal-to-noise ratio at the brainstem level may be a generator of established behavioural and neural irregularities found in poor readers. However, dyslexia is familial and moderately heritable but very little is known about the function of identified candidate genes. Knockdown of the dyslexia associated gene Kiaa0319 impairs temporal responses to speech stimuli in rat auditory pathway. We studied, whether KIAA0319 polymorphisms relate to phonological skills, phoneme discrimination, speech-in-noise perception and speech-evoked brainstem responses in a group of pre-reading children at familiar risk of dyslexia and in aged-matched control children. KIAA0319 was associated with the consistency of speech-evoked brainstem responses as well as with pseudoword repetition. KIAA0319 was not associated with speech perceptual acuity, phonological awareness, or speech-in-noise perception in pre-reading children. It remains to be shown, to what extend reading outcome will be related to the pre-reading physiological measures and to the genotype.

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Poster

683. Developmental Disorders: Other

Location: Hall A

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Program#/Poster#: 683.24/H2

Topic: C.06. Developmental Disorders

Support: MIC (TK grant)

Title: FEZ1 regulates autophagy and protects against neuropsychiatric manifestation

Authors: *A. SUMITOMO[1], K.-I. NAKAYAMA[2], A. SAWA[3], T. TOMODA[1];
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Abstract: Fasciculation and elongation protein zeta-1 (FEZ1) is a scaffolding protein that regulates neuronal development and vesicle transport. FEZ1 interacts with Disrupted-In-Schizophrenia 1 (DISC1), a susceptibility factor for major mental illnesses, such as depression and schizophrenia. Although mice deficient for Fez1 display neuropsychiatric phenotypes such as psychostimulant-induced hyperlocomotion, underlying cellular mechanisms that account for such behavioral abnormalities remain to be understood. Here we show that FEZ1 regulates autophagy by binding with LC3, an autophagic membrane protein. In response to autophagy induction, FEZ1_LC3 interaction was upregulated, as confirmed by co-immunoprecipitation and Förster resonance energy transfer (FRET) assays. This interaction was mediated in part by phosphorylation of FEZ1 by Ulk1 serine/threonine kinase, and attenuated by treatment with psychoactive substances. Consistently, neurons in prefrontal cortex of Fez1 knockout (KO) mice show elevated levels of sequestosome 1 (p62) expression, suggesting attenuated autophagy. In behavioral assays, Fez1-KO mice showed hyperlocomotion and impulsivity, which were normalized by chronic treatment with methylphenidate, a psychostimulant used to control symptoms of attention-deficit hyperactivity disorder (ADHD), or with guanfacine, an α2 adrenergic receptor agonist, which is also used to treat ADHD. These lines of evidence imply that Fez1-KO mice may serve as a new mouse model of ADHD. Moreover, lithium, a mood stabilizer with autophagy-inducing activity, significantly suppressed both hyperlocomotion and impulsivity phenotypes in Fez1-KO mice, suggesting that augmenting autophagy might serve as a novel therapeutic approach against ADHD. Additionally, HPLC analysis revealed hypo-dopaminergic status of nucleus accumbens in Fez1-KO mice, a phenotype frequently observed for ADHD patients. Effects of methylphenidate, guanfacine and lithium on dopamine contents are being evaluated after treating Fez1-KO mice with these drugs, in order to further establish the predictive validity of this mouse line as a novel model for ADHD, and also to validate the autophagy induction strategy as a novel ADHD treatment. Taken together, we propose that FEZ1 regulates autophagy and protects against neuropsychiatric manifestation.

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Poster

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Topic: C.06. Developmental Disorders

Support: Wellcome Trust

Medical Research Council

Rhodes Trust

Title: The role of dyslexia-susceptibility candidate genes Kiaa0319 and Kiaa0319-Like in brain development

Authors: *L. GUIDI[1], I. MARTINEZ-GARAY[1], Z. G. HOLLOWAY[1], M. BAILEY[1], T. SCHNEIDER[2], A. P. MONACO[1], A. VELAYOS-BAEZA[1], Z. MOLNAR[1];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Univ. of Durham, Durham, United Kingdom

Abstract: The capacity for language is a key innovation underlying the complexity of human cognition and its evolution but little is understood about the molecular and neurobiological mechanisms underlying normal or impaired linguistic ability. Developmental dyslexia is a specific impairment in reading ability despite normal intelligence, educational opportunity or major sensory defects, and it is the most common neurodevelopmental disability in school-aged children [1]. Molecular genetics studies have linked several genes to susceptibility to dyslexia and, amongst these, KIAA0319 emerges as a prime candidate based on consistently replicated associations [2], with some genetic overlap with other neurodevelopmental disorders [3]. Interestingly, the paralogous gene KIAA0319-Like is the only other member of this gene family and has also been linked to dyslexia [4]. ShRNA-mediated knockdown of the rat homologues Kiaa0319 or Kiaa0319-Like were shown to impair neuronal migration in the developing neocortex [5-8], similarly to other main dyslexia-susceptibility candidate genes [e.g. 9]. Combined with human histopathological and neuroimaging studies, these findings led to the hypothesis that dyslexia is a neuronal migration disorder [10]. We are conducting detailed analyses at the molecular, neurobiological and behavioural levels using transgenic mouse lines targeting the Kiaa0319 and/or Kiaa0319-Like loci. Neuroanatomical examination of constitutive knockouts have revealed no disruption in cortical lamination or neurogenesis. Acute knockout by *in utero* electroporation of Cre-expressing plasmids in floxed mice revealed similar results and no morphological abnormalities were detected in the hippocampus and cerebellum, other laminated brain regions. Behavioural characterisation of these mice are currently ongoing. This

data suggests these genes are not required for neuronal migration in mice, contrasting with previous findings using shRNA in rats [5-8]. This discrepancy may indicate potential methodological differences, possibly due to off target effect of shRNA [11], or be indicative of functional divergence across species. [1] Peterson, R. L. et al. Lancet 6736, 2012 [2] Carrion-Castillo, A. et al. Dyslexia 19, 2013 [3] Newbury, D. F. et al. Behav. Genet. 41, 2011 [4] Couto, J. M. et al. J. Neurogenet. 22, 2008 [5] Paracchini, S. et al. Hum. Mol. Genet. 15, 2006 [6] Peschansky, V. J. et al. Cereb. Cortex 20, 2010 [7] Platt, M. P. et al. Neuroscience 248C, 2013 [8] Adler, W. T. et al. PLoS One 8(5), e65179, 2013 [9] Meng, H. et al. Proc. Natl. Acad. Sci. U. S. A. 102, 2005 [10] Galaburda, A. M. et al. Nat. Neurosci. 9, 2006 [11] Baek, S. T. et al. Neuron 82, 2014

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Poster

683. Developmental Disorders: Other

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Topic: C.06. Developmental Disorders

Support: National Research Foundation of Korea (NRF) and the Korea Healthcare Technology Research and Development Project, Ministry for Health and Welfare Affairs, Korea (grant number A120013)

Title: A transgenic animal model of the hyperactive phenotype of ADHD

Authors: *I. I. DELA PEÑA[1], J. DELA PENA[1], H. KIM[1], I. DELA PENA[2], J. RYU[3], B.-N. KIM[4], D. HAN[5], J. CHEONG[1];
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Abstract: Attention-deficit/hyperactivity disorder (ADHD) is the commonest neurobehavioral developmental disorder of childhood. It characterized by the three core symptoms of hyperactivity, impulsivity, and inattention. The exact cause of ADHD is still unknown, but substantial evidence has shown this disorder has a significant genetic component. Genetic animal

models have been remarkably valuable in the understanding of this disorder. Although it cannot accurately reflect the human condition, animal models can yield insight into the disorder that cannot be obtained from human studies because of numerous limitations. However, ADHD is a heterogeneous disorder, and a single model is unlikely to be able to mimic all of its symptoms. At the same time, a model that recapitulates key endophenotypes can be useful in understanding the biological basis of ADHD and in developing specific pharmacological therapies for the clinical subtypes of this disorder. Thus, the present study sought to develop an animal model of ADHD which would recapitulate the hyperactive phenotype of the disorder. To explore some of the potential genetic underpinnings of hyperactivity in ADHD, we examined common differentially expressed genes (DEGs) in the prefrontal cortex of SHR/Ncrl, the most validated animal model of ADHD. In contrast with Wistar and WKY rats, a strain representing the "normal" heterogeneous population, SHR shows hyperactivity in various behavioral tasks. In addition, these symptoms were attenuated by treatment of psychostimulants. The common DEGs in the PFC of SHR vs. WKY/NCrl and Wistar rats are those involved in transcription, circadian rhythm, and corticotropin secretion. We then made transgenic animal models in which these genes are over- or under-expressed. Here we report our initial finding that mice over-expressing one of the candidate gene indeed showed hyperactive behavior. Additional studies are underway to further characterize the behavior of this new transgenic animal model of this transgenic animal and to elucidate any underlying neurobiological changes.

Disclosures: I.I. dela Peña: None. **J. dela Pena:** None. **H. Kim:** None. **I. dela Pena:** None. **J. Ryu:** None. **B. Kim:** None. **D. Han:** None. **J. Cheong:** None.

Poster

683. Developmental Disorders: Other

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 683.27/H5

Topic: C.06. Developmental Disorders

Title: Altered regional homogeneity in children with ADHD: A surface-based analysis

Authors: *J. YOO[1], I. KIM[3], B.-N. KIM[4], B. JEONG[2];
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Abstract: Introduction Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders of childhood. Previous studies have implicated that

dysfunctional modulation of the default mode network activity might be responsible for regulation deficits in children with ADHD. We hypothesized that spontaneous brain activity of ADHD can be affected by either disease state or developmental change with aging. For this aim, we tested functional measures reflects spontaneous brain activation such as amplitude of low frequency fluctuation (ALFF), fractional ALFF (fALFF), and regional homogeneity (ReHo) with resting state functional MRI (rs-fMRI). **Methods** A total of 181 subjects (120 ADHD, 61 Control), were included in this study. ALFF and fALFF were calculated using frequency band around 0.009-0.08 Hz and ReHo was measured from Kendal's coefficient of concordance from 27 surrounding voxels. For the accurate registration, those measures projected to spherical surface created by Freesurfer image analysis suite version 5.3 (http://surfer.nmr.mgh.harvard.edu/), then, the effect of diagnosis and interaction with age were estimated using general linear model after controlling effect of gender, IQ and intracranial volume. **Results** ADHD group showed reduced ReHo in the cluster located at the left postcentral ($z=-6.319$, $p=0.002$) and right superior temporal surface ($z=-7.3685$, $p=0.002$). In contrast, a cluster in precentral area had increased ReHo than healthy control group ($z=5.711$, $p=0.002$). Significant group effect was not found in ALFF and fALFF analysis, and none of the measures showed age by diagnosis interaction effect. **Discussion** We found alteration of ReHo in primary sensory and motor cortex in ADHD group, which suggests baseline spontaneous neuronal activity of these regions reflects underlying pathophysiology in children with ADHD.

Disclosures: J. Yoo: None. **I. Kim:** None. **B. Kim:** None. **B. Jeong:** None.

Poster

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Topic: C.06. Developmental Disorders

Support: NIH Grant R15DA029544

The Rita Levi-Montalcini IBRO Grant

Title: Goal-directed behavior is impaired in Spontaneously Hypertensive Rats and Methylphenidate remediates this deficit in a dose-dependent manner

Authors: *J. Y. NATSHEH[1,2], F. AL-SHAMMARY[3], A. KOSC[3], M. W. SHIFLETT[3];
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Abstract: Attention Deficit Hyperactivity Disorder (ADHD) is a psychiatric disorder that is primarily characterized by symptoms of inattention. Attentional impairments are well described in ADHD; however, no studies have examined goal-directed control over behavior. In a previous study, using outcome devaluation paradigm, we examined goal-directed behavior and the effect of methylphenidate (MPH) on the pattern of this behavior in adult (P75-P105) Spontaneously Hypertensive Rat strain. SHR is the best validated animal model of ADHD as it mimics the fundamental behavioral, genetic and neurobiological correlates of ADHD in humans. We found that SHR has a deficit in goal-directed behavior as compared to Wistar Kyoto controls (WKY). Administration of 2.0 mg/kg of MPH remediated this deficit in SHR and impaired goal-directed behavior in WKY. To validate our findings, we performed three follow-up experiments: (1) we tested the same age group of SHR and WKY on a contingency degradation test, which provides an alternative assessment of goal-directed behavior; (2) we tested adolescent (P35-P60) SHR and WKY rats on outcome devaluation paradigm to characterize the pattern of goal-directed behavior in a different age group; (3) we investigated the effects of different doses of MPH (0.5, 1.0, and 4.0 mg/kg) on goal-directed behavior in adolescent SHR and WKY rats. In all experiments we trained the rats on an instrumental conditioning paradigm in which they separately acquired two distinct action-outcome contingencies. In Experiment 1, we degraded one of the acquired action-outcome contingencies by non-contingently delivering one of the instrumental outcomes. During an extinction test SHR showed no effect of contingency degradation on lever preference as compared to WKY that reduced responding on the degraded lever. In Experiment 2, we tested adolescent SHR and WKY rats on a choice test conducted in extinction following instrumental training. Prior to test one of the food outcomes was devalued through specific satiety and rats received a dose of either 2.0 mg/kg of MPH or normal saline. SHR showed no effect of outcome devaluation on choice behavior, in contrast to WKY. Furthermore, unlike adult SHR rats, a dose of 2.0 mg/kg did not restore this deficit. In Experiment 3, we used the same paradigm in experiment 2, but with different doses of MPH. We found that only a dose of 1.0 mg/kg of MPH is sufficient to restore outcome-sensitive behavior in this age group. These experiments strongly suggest that SHR has a fundamental deficit in goal-directed action control that can be remediated by MPH administration in a dose-dependent manner according to the animal's developmental stage.

Disclosures: J.Y. Natsheh: None. **F. Al-Shammary:** None. **A. Kosc:** None. **M.W. Shiflett:** None.

Poster

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Topic: C.06. Developmental Disorders

Support: NIH Grant MH099709

Title: New EEG measures reveal infra-slow fluctuations in both attending and ignoring in adults with ADHD that provide high accuracy in discriminating ADHD from control

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Abstract: There is a great need for brain-based measures in the assessment of Attention Deficit/Hyperactivity Disorder (ADHD) for better diagnosis and treatment evaluation, and for development of brain-based models (in keeping with RDoC). Within-individual variability in performance is commonly cited as reflecting inconsistent control of attention in ADHD, and has been characterized as infra-slow fluctuations in performance (roughly 0.1 to 0.02Hz). We also know that attention control involves two separable functions (and possibly brain systems), attending and ignoring, suggesting that ADHD might involve deficits in consistent and purposeful maintenance of either attending or ignoring or both. We created and tested a new EEG-based method for characterizing the infra-slow fluctuations (ISFs) of steady-state visual evoked potentials (SSVEPs) reflecting attended target and ignored distractor processing during a sustained attention task. We studied 28 adults with ADHD and 28 matched controls with a battery of neuropsychological tests, questionnaires and cognitive tests as well as the new EEG measure - the Neurophysiological Attention Test (NAT). The NAT uses a sustained spatial attention task in which the stimuli on the left and right of fixation flicker at different frequencies providing SSVEPs corresponding to the attended and ignored stimuli. We analyzed the infra-slow fluctuations in the SSVEP magnitudes (256 channel recordings) for both attending and ignoring over time during the sustained attention task. Consistent with our hypothesis, the results revealed that adults with ADHD have much larger infra-slow fluctuations in both their attended and ignored SSVEP signals as well as their performance relative to control participants. Furthermore, the NAT indicators, both behavioral and EEG, successfully discriminated between individuals with ADHD from healthy comparison individuals (effect size = 0.75), and correlated with scores on rating scales widely used to characterize the inattentive and impulsive features of ADHD. Discriminant function analyses revealed the NAT to be superior to the Conners CPT and the Theta/Beta EEG measures: Sensitivity: 79%, 79% and 43% (respectively) and Specificity: 89%, 82%, 64% (respectively). We conclude that attention control in ADHD is associated with

instability in the sustaining of attending and ignoring processes, this instability can be measured using the newly developed NAT, which offers high accuracy in predicting diagnosis.

Disclosures: **A. Lenartowicz:** None. **G.V. Simpson:** A. Employment/Salary (full or part-time):; Think Now, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Think Now, Inc. **S.R. O'Connell:** A. Employment/Salary (full or part-time):; Think Now, Inc. **S.L.M. Noah:** A. Employment/Salary (full or part-time):; Think Now, Inc.. **A.L. Head:** None. **R.M. Bilder:** None. **J.T. McCracken:** None. **S.Y. Bookheimer:** None. **R. Reid:** None. **M.S. Cohen:** None.

Poster

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DoD CDMRP, W81XWH-12-1-0187

Title: Characterizing the effects of Tsc1 mutations on thalamic circuit function

Authors: R. MARTINEZ-GARCIA, J. M. SMITH, S. R. CRANDALL, B. VOELCKER, M. ZERVAS, *B. W. CONNORS;
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Abstract: Tuberous Sclerosis (TSC) is a developmental genetic disorder caused by mutations in *TSC1* and/or *TSC2*. Neurocognitive signs in TSC patients include epilepsy, intellectual disability, and autism, but the altered cellular mechanisms underpinning TSC are poorly understood. One brain region implicated in the pathology of some TSC patients is the thalamus. Thalamic relay nuclei are functionally distinct clusters of excitatory neurons that project to the neocortex. Relay neurons also excite neurons of thalamic reticular nucleus (TRN), which in turn inhibit relay neurons. The laboratory previously generated a mouse model in which *Tsc1* was deleted in ~70% of relay neurons on E12.5 (Normand et al., *Neuron*, 78:895, 2013). This mosaic deletion was sufficient to disrupt thalamic circuits and caused aberrant repetitive grooming and seizures. An

unresolved question is how functional neural circuits are affected in this model. We tested whether mosaic *Tsc1* deletion alters the intrinsic properties, synaptic function, and synaptic architecture of thalamic relay neurons. We first replicated previous results (Normand et al., 2013), confirming that $Tsc1^{\Delta/\Delta(Thal)}$ relay neurons have lower input resistance and reduced excitability. Action potentials of $Tsc1^{\Delta/\Delta(Thal)}$ neurons have larger amplitudes and faster rates of depolarization and repolarization. When firing in tonic mode, $Tsc1^{\Delta/\Delta(Thal)}$ neurons have shallower frequency vs. stimulus current slopes than those of wild type relay neurons. When firing in bursting mode, $Tsc1^{\Delta/\Delta(Thal)}$ neurons have increased intra-burst frequency compared to wild type relay neurons. $Tsc1^{\Delta/\Delta(Thal)}$ neurons also have ectopic expression of the calcium binding protein parvalbumin, which may affect thalamic physiology (Normand et al., 2013). Interestingly, *Tsc1* deletion in relay neurons decreased the frequency of miniature excitatory postsynaptic currents and reduced the paired pulse depression of evoked synaptic responses in neurons of the TRN. Our results suggest that altered neurotransmission is likely caused by changes in presynaptic function, such as reduced release probability. Circuit dynamics can also be altered by changing the number and distribution of synapses. However, we observed no significant differences in excitatory or inhibitory synaptic markers in relay neurons or excitatory synaptic markers in TRN neurons. We are now testing how mosaic deletion of *Tsc1* in relay neurons affects synaptic strength, dynamics, and network functions of the relay neuron-TRN circuit.

Disclosures: **R. Martinez-Garcia:** None. **J.M. Smith:** None. **S.R. Crandall:** None. **B. Voelcker:** None. **M. Zervas:** None. **B.W. Connors:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: NIMH Grant R01-MH087583

NIMH Grant R01-MH099085

NIMH Grant R01-MH058616

Title: Acute prenatal exposure to valproic acid (VPA) alters social and anxiety-like behaviors in prairie voles

Authors: *L. L. ELVIR[1], H. WANG[2], F. DUCLOT[1], Y. LIU[2], Z. WANG[2], M. KABBAJ[1];
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Abstract: Previous studies have shown that rats and mice prenatally treated with sodium valproate (valproic acid, VPA) exhibit deficits in social behaviors that resemble some aspects of autism spectrum disorders. In this study, the socially monogamous prairie vole *(Microtus ochrogaster)* was used as a novel animal model to examine social behaviors following prenatal VPA exposure. Male and female control and VPA-exposed subjects were assessed on a battery of social tests to evaluate the VPA-induced social deficits and anxiety-like behavior. VPA-pretreated voles engaged in fewer play behaviors and had reduced social interaction with novel conspecifics of the same age, compared to control animals. VPA-pretreated male, but not female, subjects showed enhanced anxiety-like behavior and did not develop partner preference when they became adults. We are now in the process of examining whether some of these VPA-induced social deficits in male subjects can be ameliorated by histone deacetylase (HDAC) inhibitors administered at different developmental periods.

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Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: ISF Grant 225/11

EU Grant PCIG9-GA-2011-294001

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Title: Functional connectivity impairments resulting from neurofibromin deficiency are linked to striatal dysfunction

Authors: *B. SHOFTY[1], E. BERGMANN[1], N. COHEN[1], A. KAVUSHINSKY[1], S. CONSTANTINI[2], I. KAHN[1];
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Abstract: Deletion of neurofibromin, results in several cellular and systemic defects. Children suffering from this syndrome (termed neurofibromatosis type 1 [NF1]) display a unique profile of attention, memory and various psychomotor impairments. A potential pivotal role for the basal ganglia in NF1 associated impairments was suggested. We used whole-brain intrinsic functional connectivity MRI to interrogate functional changes in NF1 mice. Three Nf1+/- mice were scanned awake in a 9.4 Tesla MRI, for a total of 116 scans (15.4 data hours), and 3 littermate Nf1+/+ (wild-type) mice serving as control animals were scanned a total of 120 scans (16 data hours). Coherent slow fluctuations in the fMRI signal across the entire brain were used to interrogate the pattern of functional connectivity of the cortical and subcortical structures between NF1 and wild-type mice and as compared to normal connectional anatomy in the mouse brain. While neurofibromin deficiency is estimated to be equivalent across cortical regions, measures of functional connectivity differed across brain systems. Specifically, primary motor cortex demonstrated reduced correlation with contralateral primary motor and secondary motor cortices, resulting in overall reduced motor cortical network correlations. Primary somatosensory cortex showed increased correlation with contralateral primary somatosensory, and secondary somatosensory cortices, resulting in overall increased somatosneosry cortical network correlations. Brain-wide analysis revealed a common source for this effect when comparing the projection fibers of these regions to subdivisions within the dorsal striatum. In both pathways increased corticostriatal connectivity was observed in the NF1 group. While maintaining spatial organization in relation to connectional anatomy, the NF1 group demonstrated increased corticostriatal connectivity with both motor and sensory cortices. Next we sought to test whether this pattern of functional connectivity was present in NF1 pediatric patients. We performed whole-brain intrinsic functional connectivity MRI in NF1 pediatric patients (n = 20; 6.2 ± 2.8 yo) and age-matched controls (n = 20; 7.7 ± 0.6, yo). Similar to the NF1 animals, we found that corticostriatal connectivity is increased in the patient group when compared to normal controls. The present results implicate the dorsal striatum as a source for altered cortical activity seen in NF1 patients. We would like to propose the striatum as a therapeutic target for alleviating the neurocognitive phenotype in neurofibromatosis type 1.

Disclosures: **B. Shofty:** None. **E. Bergmann:** None. **N. Cohen:** None. **A. Kavushinsky:** None. **S. Constantini:** None. **I. Kahn:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: T32GM007280 (E.N.)

NINDS5T32DA007135-29 (M.D.)

MCHDI (H.M.)

Title: Adolescent suppression of prefrontal nicotinic signaling shapes attention

Authors: *E. NABEL, M. DEMARS, J. SHORT, H. KOIKE, H. MORISHITA;
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Abstract: Attention, the cognitive function that selects task-relevant information, is pervasively impaired in neurodevelopmental disorders and psychiatric illnesses with developmental contributions. Despite this important link, the mechanisms driving development in neural systems mediating attention are poorly understood. Regulatory mechanisms of visual cortex development -the preeminent model for cortical maturation- have been identified in other brain regions and provide a conceptual framework for studying cortical development underlying other functions. Lynx1, a critical period regulator, spurs cortical maturation in the visual cortex by antagonizing nicotinic acetylcholine receptors (nAChRs). Furthermore, the nACh system is integral to attention processing. In light of this converging evidence, we tested the hypothesis that Lynx1 plays a key role in establishing frontal cortex-dependent attentional function. We coupled genetic, viral, pharmacological, and histological approaches to behavioral measures of attention using a translational automated touchscreen version of the 5 choice serial reaction time test. We first found that Lynx1 knock-out mice displayed attention deficits in adulthood. This functional deficit was associated with reduced task-dependent-activation of anterior cingulate cortex (ACC) neurons that increasingly express Lynx1 during the peri-adolescence period and throughout adulthood. Viral knockdown of Lynx1 in the ACC from adolescence into adulthood phenocopied the impairment. Strikingly, this attention deficit was rescued by a chronic 10 day pharmacologic nAChR blockade both during peri-adolescent and adult periods -but not by acute blockades during attention testing. These data suggest that, in the absence of Lynx1, excess nAChR signaling across adolescent development may “freeze” attentional circuit maturation, rendering immature cortical circuits that underlie aberrant frontal cortex activity and long-lasting impairment in attention. Developmental regulation of attentional function by Lynx1 may prove a novel mechanism and therapeutic target for a major cognitive function disturbed in psychiatric disorders with developmental contributions including autism, ADHD, and schizophrenia. *E.N., M.D., J.S. equal contribution

Disclosures: E. Nabel: None. **M. Demars:** None. **J. Short:** None. **H. Koike:** None. **H. Morishita:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: CONACyT scholarship 212825 (to AAPL)

Title: Brain tissue and plasma amino acid concentration in developing rats prenatally exposed to valproic acid

Authors: *A. PUIG-LAGUNES[1], L. ROCHA[2], A. PUIG-NOLASCO[3], I. ZAMORA-BELLO[1], E. VELAZCO-CERCAS[1], R. MEDEL-MATUS[1], J.-S. MEDEL-MATUS[4], J. MANZO[1], R. TOLEDO CÁRDENAS[1], M.-L. LÓPEZ-MERAZ[1];
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Abstract: Evidence supports that autism spectrum disorder (ASD) is associated with modifications in GABAergic and glutamatergic neurotransmission, effect that may be related to hiperexcitability observed in those patients. In fact, some epidemiological reports show higher prevalence of epilepsy (10-30%) in ASD patients than in the general population. The aim of this study was to determine brain tissue and plasma amino acid concentration in rats prenatally exposed to valproic acid (VPA), a well-accepted experimental model of autism. Pregnant females were injected with VPA (600 mg/Kg, i.p.) during the twelfth embryonic day (E12); control rats were injected with saline solution during E12. On the fourteen postnatal day, rats from both experimental groups were anesthetized with pentobarbital, euthanized by decapitation and their brain dissected out on frappe ice. Then, frontal cortex, hippocampus, amygdala, brain stem and cerebellum were dissected and homogenized in 0.1 M perchloric acid containing 4 mM sodium bisulfite. Homogenates were centrifuged and supernatant was used to quantify GABA, glutamate, glutamine, alanine and glycine tissue concentrations by HPLC coupled with fluorometric detection. Blood samples were obtained by cardiac puncture on anesthetized rats; plasma was separated and deproteinized with 0.5 M perchloric acid in order to quantify amino acids concentration by HPLC. Amino acid tissue levels were similar in control and VPA rats for all the brain areas analyzed. Plasma concentration of GABA was significant higher (53%) in VPA rats when compared with control rats ($t=4.04$, $p=0.0004$); however, no differences were detected between experimental groups for the other amino acids evaluated. Increased GABA concentration observed in the plasma from rats prenatally exposed to VPA could be related to brain immaturity and hiperexcitability due to an excitatory effect of GABA.

Disclosures: **A. Puig-Lagunes:** None. **L. Rocha:** None. **A. Puig-Nolasco:** None. **I. Zamora-Bello:** None. **E. Velazco-Cercas:** None. **R. Medel-Matus:** None. **J. Medel-Matus:** None. **J. Manzo:** None. **R. Toledo Cárdenas:** None. **M. López-Meraz:** None.

Poster

684. Developmental Disorders: Animal Models I

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: NIH Grant R01MH080055

Title: Experience-dependent morphological changes in the indirect basal ganglia pathway mediate repetitive motor behaviors

Authors: A. BECHARD[1], N. CACODCAR[2], M. A. KING[3], A. MUEHLMANN[4], *M. H. LEWIS[4];
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Abstract: Repetitive motor behaviors are observed in many neurodevelopmental and neurological disorders (e.g. autism, Tourette syndrome, fronto-temporal dementia). Despite their clinical importance, the neurobiology underlying these invariant, apparently functionless behaviors is poorly understood, and there are no effective pharmacological treatments. Identification of mechanisms that mediate the development of repetitive behaviors will aid in the discovery of new therapeutic targets and treatment development. Using a deer mouse model, we have shown that decreased indirect basal ganglia pathway activity is associated with high levels of repetitive behavior. We, and others, have shown that environmental enrichment (EE) markedly attenuates the development of such aberrant behaviors in mice, although mechanisms driving this effect are unknown. Thus, we hypothesized that EE would reduce repetitive behavior by increasing indirect basal ganglia pathway function. We tested the effects of EE on repetitive behavior of deer mice and basal ganglia dendritic morphology. Following Golgi-Cox histochemistry, we measured dendritic spine density and length in the dorsolateral striatum (DLS), substantia nigra pars reticulata (SNR), and subthalamic nucleus (STN) in adult mice reared in EE and standard housing. EE significantly increased dendritic spine density only in the STN and only for those mice that exhibited a marked EE-induced decrease in repetitive behavior. As the STN lies exclusively in the indirect pathway, these data suggest that EE-induced attenuation of repetitive behavior is associated with increased functional activation of the indirect

basal ganglia pathway. These results are consistent with our other findings highlighting the importance of the indirect pathway in mediating repetitive behaviors.

Disclosures: **A. Bechard:** None. **N. Cacodcar:** None. **M.A. King:** None. **A. Muehlmann:** None. **M.H. Lewis:** None.

Poster

684. Developmental Disorders: Animal Models I

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 684.06/H14

Topic: C.06. Developmental Disorders

Title: Novel murine models of creatine transporter deficiency

Authors: ***L. BARONCELLI**[1], M. ALESSANDRÌ[2], J. TOLA[1], E. PUTIGNANO[1], D. NAPOLI[1], M. MIGLIORE[1], E. AMENDOLA[3], F. ZONFRILLO[3], C. GROSS[3], M. MAZZANTI[4], V. LEUZZI[5], G. CIONI[2,6], T. PIZZORUSSO[1,7];
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Abstract: Mutations in the creatine (Cr) transporter (CrT) gene lead to cerebral creatine deficiency syndrome-1 (CCDS1), an X-linked metabolic disorder characterized by cerebral Cr deficiency causing intellectual disability, seizures, movement and behavioral disturbances, language and speech impairment (OMIM #300352). CCSD1 is still an untreatable a pathology that can be very invalidating for patients and caregivers. Only two murine models of CCDS1, one of which is an ubiquitous knockout mouse, are currently available to study the possible mechanisms underlying the pathologic phenotype of CCSD1 and to develop therapeutic strategies. Given the importance of validating phenotypes and the efficacy of promising treatments in more than one mouse model we have generated a new murine model of CCSD1 obtained by ubiquitous deletion of 5-7 exons in the Slc6a8 gene. We showed a remarkable Cr depletion in the murine brain tissues and cognitive defects, thus resembling the key features of human CCSD1. These results confirm that CCDS1 can be well modeled in mice. This CrT−/y

murine model will provide a new tool for increasing the relevance of preclinical studies to the human disease.

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Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: NIH R01 NS35714

Title: Generation and characterization of a CNS-specific Ikbkap/Elp1 mouse model of Familial Dysautonomia

Authors: ***M. A. MERGY**[1], M. CHAVERRA[1], L. GEORGE[1], N. PODGAJNY[1], H. WALLER[1], A. GRINDELAND[2], C. CUSICK[1], G. CARLSON[2], F. LEFCORT[1];
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Abstract: Familial Dysautonomia (FD), also known as Hereditary Sensory and Autonomic Neuropathy (HSAN) Type III, results from an intronic point mutation (IVS20 +6T → C) in the gene IKBKAP. Classic hallmarks of the disease include decreased pain and temperature sensation, orthostatic hypotension, scoliosis, reduced tear production, and dysautonomic "crises" marked by vomiting, hypertension, and tachycardia. In addition to substantial changes to the peripheral nervous system (PNS), the central nervous system (CNS) is also abnormal in FD patients. CNS signs of FD include high anxiety, learning and cognitive impairment, seizures, decreased motor nerve conduction, ataxia, and reductions in size in specific CNS nuclei. We have previously reported a mouse model in which Ikbkap is selectively deleted from neural crest cells (precursors for most of the PNS) and found that neural crest migration occurs normally, and that Ikbkap is required for survival of neural progenitors and post-mitotic neurons in the PNS (Hunnicutt et al., 2012; George et al., 2013; Jackson et al., 2014). We have subsequently generated a mouse model where Ikbkap is selectively deleted from the CNS (cKO). This mouse model recapitulates several characteristics of human FD, including small stature, kyphosis, and slow gait. Gross morphological analysis reveals that the brains of Ikbkap cKO mice are smaller

than control brains, and show enlarged lateral ventricles and hippocampi (Chaverra et al., in preparation). We have also examined behavioral alterations in our Ikbkap cKO mice and have found a non-significant increase in basal locomotor activity, a significant reduction in anxiety-like behavior, and apparent learning and memory deficits. Furthermore, our cKO mice display repetitive jumping behavior and abnormal social interactions with conspecific mice, traits commonly associated with autism spectrum disorders. Our CNS-specific Ikbkap knockout mouse displays many features of FD and will be a valuable tool for studying the role of the CNS in this disease.

Disclosures: M.A. Mergy: None. **M. Chaverra:** None. **L. George:** None. **N. Podgajny:** None. **H. Waller:** None. **A. Grindeland:** None. **C. Cusick:** None. **G. Carlson:** None. **F. Lefcort:** None.

Poster

684. Developmental Disorders: Animal Models I

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 684.08/H16

Topic: C.06. Developmental Disorders

Support: NINDS5T32DA007135-29 (M.D.)

MCHDI (H.M.)

Title: Chemogenetic inactivation of dorsal anterior cingulate cortex neurons disrupts attentional behavior in mouse

Authors: *H. KOIKE[1], M. DEMARS[1], J. SHORT[1], E. NABEL[1], S. AKBARIAN[1,2,3], M. BAXTER[1,2,3], H. MORISHITA[1,2,4,5,3];
[1]Psychiatry, [2]Neurosci., [3]Friedman Brain Inst., [4]Ophthalmology, [5]Mindich Child Hlth. and Develop. Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Attention, the process of selecting task-relevant information from incoming sensory stimuli, is disrupted commonly in psychiatric disorders. Dorsal anterior cingulate cortex (dACC) excitotoxic lesions and pharmacological disinhibition are associated with deficits in this cognitive ability, however, no causal link between dACC neuronal activity and attention performance has been established. Moreover, this association has been observed in humans, monkey, and rat, but has not yet been examined in a genetically tractable species such as mice. Here, we establish a causal role of the dACC neuronal activity in attention behavior by

combining a chemogenetic approach that allows reversible neural activity manipulations with a translational, touchscreen-based attention task. We virally expressed inhibitory hM4Di designer receptor exclusively activated by a designer drug (DREADD) in dACC neurons, and examined the effects of this inhibitory action with the attention-based 5-choice serial reaction time task. DREADD inactivation of the dACC neurons during the task significantly increased omission and correct response latencies, indicating that the neuronal activities of dACC contribute to attention and processing speed. Selective inactivation of excitatory neurons in the dACC not only increased omission, but also decreased accuracy. This finding suggests that dACC excitatory neurons play a principle role in modulating attention and further proposes that a physiologic balance between excitatory and inhibitory tone is pertinent to attention behavior. The effect of inactivating dACC neurons was selective to attention as response control, motivation and locomotion remain normal. Our findings demonstrate that dACC neuron inactivation produces attention deficits. This study establishes a foundational chemogenetic approach in a genetically tractable species to dissect specific cell-type and circuit mechanisms underlying attentional behaviors.

Disclosures: **H. Koike:** None. **M. Demars:** None. **J. Short:** None. **E. Nabel:** None. **S. Akbarian:** None. **M. Baxter:** None. **H. Morishita:** None.

Poster

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Topic: C.06. Developmental Disorders

Support: DA027487

MH083807

Title: Neural activation differences in prefrontal-striatal-cerebellar circuitry and striatal gene expression in a selectively bred hyperactive mouse line

Authors: ***P. MAJDAK**[1], B. PANOZZO[2], E. L. GROGAN[2], T. K. BHATTACHARYA[2], J. S. RHODES[2];
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Abstract: Attention deficit-hyperactivity disorder (ADHD) is a relatively common behavioral disorder characterized by developmentally inappropriate levels of hyperactivity, inattention and

impulsivity. It is highly heritable; however, the specific genes and neurobiological risk factors remain unknown, partly due to the fact that few animal models have been generated for the purpose of exploring the neurobiological changes underlying a hyperactive phenotype. The goal of this study was to continue our ongoing evaluation of a line of mice selectively bred for increased locomotor activity in the home cage for construct validity as an animal model for ADHD. Two lines of mice were maintained for over 18 generations. One line, referred to as High-Active, was subjected to within family selection each generation for increased total distance traveled in the home cage. The other line, referred to as unselected Control, was randomly bred each generation (except avoiding sibling mating). The purpose of the first experiment was to examine whether High-Active mice show differential activation of the prefrontal cortex, striatum, and cerebellum as compared to Control mice under baseline conditions. Reduced functionality of these regions is increasingly identified in clinical observations of individuals with ADHD. Neural activation in these lines was assessed by immunohistochemical detection of the protein product of the immediate early gene, c-fos. Furthermore, we were interested in determining whether therapeutic amphetamine administration would correct regional deficits in the High-Active mice. Therefore mice of each line were injected with either 0.25 mg/kg amphetamine or saline 90 minutes prior to sacrifice. The purpose of the second experiment was to determine via qPCR whether certain candidate genes identified as contributors to the pathophysiology of ADHD (dopamine- and serotonin-related) are differentially expressed in the striatum of our High-Active vs. Control mice. Preliminary data suggest that amphetamines differentially regulate prefrontal cortical activation of High-Active mice relative to Controls, which supports the construct validity of our High-Active line as a potential model for ADHD.

Disclosures: **P. Majdak:** None. **B. Panozzo:** None. **E.L. Grogan:** None. **T.K. Bhattacharya:** None. **J.S. Rhodes:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: NIH Grant R01DA021801

The Anesthesia Research Distinguished Trailblazer Award

Title: Enhanced ascending serotonergic system activity in the Timothy syndrome mouse model of autism spectrum disorder

Authors: *D. G. EHLINGER, C. M. PANZINI, K. G. COMMONS;
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Abstract: Accumulating evidence suggests that altered function of the ascending serotonergic system may underlie the development and/or behavioral impairments characteristic of autism spectrum disorder (ASD). However, dissecting the neural underpinnings of ASD has proven to be a unique challenge for neuroscience research, as animal models must accurately represent the pervasiveness of the disorder across a widespread variety of behaviors and incorporate a known environmental or genetic influence that has been found in the human condition. The TS2-neo mouse model of ASD contains a G406R mutation in exon 8 of the CaV1.2 L-type calcium channel locus (Cacna1c) associated with Timothy Syndrome. In heterozygous mice, a proportion of CaV1.2 channels display reduced channel inactivation, leading to behavioral abnormalities reminiscent of all components in the core triad of ASD symptomology: repetitive and perseverative behavior, deficits in communication ability, and impaired social behavior. Interestingly, human genetic variation in CaV1.2 channels has also been strongly associated with neuropsychiatric illness including bipolar disorder and schizophrenia. Here, we utilize the TS2-neo mouse model to assay abnormalities in serotonergic system structure and function that may be involved in the etiology of ASD and other disorders. In response to an acute stressor known to alter the activity state of the ascending serotonin system (forced-swim test), immunofluorescent co-labeling of Tph2 and c-Fos reveals that TS2-neo mice exhibit enhanced serotonin neuron activity and 5HT1A-dependent feedback inhibition across multiple subregions of the dorsal raphe nucleus, while coping behavior remains unimpaired. These changes in serotonin neuron activity are accompanied by increased serotonin concentration and altered serotonin turnover in forebrain structures, including the dorsal striatum and amygdala, that have been previously associated with behavioral impairments in ASD, bipolar disorder, and schizophrenia. Collectively, our results suggest the presence of abnormalities in the ascending serotonergic system of TS2-neo mice, and may reflect a common neural circuit that is disrupted in both ASD and neuropsychiatric illness.

Disclosures: D.G. Ehlinger: None. **C.M. Panzini:** None. **K.G. Commons:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: Faculty Research and Creative Activities Support Fund, Western Michigan University

Title: Ectopic sox2 cells and neuroglial heterotopia in the hindbrain of the frog model of fetal alcohol syndrome

Authors: *B. KATBAMNA[1], T. BEEBE[1], C. IDE[2];
[1]Speech Pathology & Audiol., [2]Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: Previous work in our laboratory showed that developmental exposure to alcohol produced significant eye and craniofacial anomalies in the Xenopus model of fetal alcohol syndrome. The most notable abnormality associated with craniofacial malformations was ectopic positioning of ventricular zone connexin 43 (Cx43) cells in the hindbrain, with clusters in the lateral gray matter and neuropil. In the present study we examined Sox2 and BLBP expression in the proliferating cells of the ventricular zone and its relationship to Cx43 cells. Xenopus laevis embryos were exposed to 2% ethanol in spring water along with a control cohort with no ethanol, before neural tube closure at stages 10-12 for 24 hours. BrdU exposure was conducted on day 4 post-exposure for 24 hours and animals were fixed on day 5 post-exposure for assays. All animals in the control group showed BrdU and Sox2 or BLBP co-labeling at the ventricular zone, with Cx43 cells in mid-rhombomeric locations. Alcohol exposed animals showed normal overall organization of hindbrain rhombomeres, however, positioning of BrdU, Sox2 and Cx43, but not BLBP expressing cells was altered. Specifically, proliferating cells occurred at both the ventricular margin as well as in the subventricular zone. Some of these ectopic subventricular proliferating cells were also Sox2 and Cx43 positive, but not BLBP positive. Thus, alcohol exposure appears to cause abnormal movement of Sox2 and Cx43 positive proliferating cells away from the ventricular margin in the hindbrain, disrupting normal craniofacial development. Since Cx43 expressing cells have been shown to be involved in cell migration and axonal guidance during normal development, abnormal movement of Cx43 cells may explain ethanol induced neural and craniofacial abnormalities. Moreover, positional displacement of Sox2, a universal marker of neural stem cells known to promote cell division and differentiation, suggests that Sox2 may be the primary source of heterotopia related defects. In summary, the normal expression of BLBP, a stem cell marker associated with radial glial cells, but aberrant disposition of Cx43 and Sox2 in the lateral gray matter and neuropil, supports the idea that Cx43 and Sox2 may mediate heterotopia.

Disclosures: B. Katbamna: None. **T. Beebe:** None. **C. Ide:** None.

Poster

684. Developmental Disorders: Animal Models I

Location: Hall A

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Program#/Poster#: 684.12/H20

Topic: C.06. Developmental Disorders

Title: Characterization of BTBR $T^{+tf/J}$ mouse model for autism spectrum disorder

Authors: J. PUOLIVÄLI, J. OKSMAN, T. HEIKKINEN, R. PUSSINEN, *S. SAARIO, K. LEHTIMÄKI, A. NURMI;
Charles River Discovery Services, Kuopio, Finland

Abstract: Autism spectrum disorders (ASD) are a severe neurodevelopmental disorders, which typically emerge early in childhood. Animal models of ASD include various genetically modified strains as well as inbred strains of mice expressing traits relevant to autism and targeted mutations in candidate genes. One of the used models is a BTBR $T^{+tf/J}$ mouse model which reportedly exhibits several symptoms of ASD, including reduced social interactions, low exploratory behavior, unusual vocalizations, and high anxiety as compared to other inbred strains. Also, mouse brain anatomy in this model has previously revealed profound abnormalities in the mouse phenotype when compared to regular strains, such as C57Bl/6. Due to the reported ASD related features of this strain, our objective was to characterize BTBR $T^{+tf/J}$ (BTBR) model phenotype. We sought to evaluate behavioral phenotype but also brain abnormalities at different ages of the mice by using different imaging tools. BTBR and C57BL6/J mice were behaviorally tested for spontaneous locomotor activity in open field, anxiety like behavior in elevated plus maze, impairments in social behavior and interaction, cognitive functions in T-maze reversal learning and contextual fear conditioning, social transmission of food preference, as well as for stereotypic behavior at different ages. Brain anatomical and metabolic profile was assessed by using MRI and 1H-MRS, respectively. Furthermore, histological/immunohistochemical and biochemical tools were used to assess ASD related markers and to compliment MRI/1H-MRS data from the model.

Disclosures: J. Puoliväli: None. **J. Oksman:** None. **T. Heikkinen:** None. **R. Pussinen:** None. **S. Saario:** None. **K. Lehtimäki:** None. **A. Nurmi:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: Autism Speaks [Postdoctoral Fellowship] (#8679)

Intramural Research Program National Institute of Mental Health

Title: Diminished rates of protein synthesis in a mouse model of Tuberous Sclerosis Complex: an mTORC1-dependent phenomenon

Authors: *R. M. REITH, T. HUANG, T. BURLIN, C. BEEBE SMITH;
Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Tuberous Sclerosis Complex (TSC) is an autosomal dominant neurogenetic disorder affecting about 1 in 6,000 people, leading to benign growths throughout the brain and other vital organs. TSC usually has effects on the central nervous system manifested by a high incidence of seizures, intellectual disability, and autism. TSC is caused by mutations in either TSC1 or TSC2, which encode for proteins that form a complex and interact with a small GTP-binding protein, RHEB, to inhibit mammalian target of rapamycin complex 1 (mTORC1). mTORC1 is a central regulator of ribosomal biogenesis and translation initiation, and loss of TSC1/2 function results in increased activity of mTORC1. Nevertheless, heterozygous loss of Tsc2 leads to diminished protein synthesis both *in vitro* and *in vivo*. Specifically, with the *in vivo* quantitative autoradiographic L-[1-14C]leucine method, we found reduced rates of cerebral protein synthesis (rCPS) in Tsc2+/- mice at 3 months of age, particularly in the parietal cortex, dorsal and ventral hippocampus, median raphe, and visual cortex. We treated mice with rapamycin to determine if we could reverse this phenotype. We performed both acute and chronic rapamycin studies. For acute studies, we injected 3mg/kg rapamycin or vehicle (about 5% ethanol) i.v., 30 min prior to tracer infusion. For chronic studies, we treated mice from P21 until P90-P105 with rapamycin-enriched chow to achieve a daily dose of approximately 2.2mg/kg. Preliminary results indicate that acute rapamycin treatment of Tsc2+/- mice (n=5) increased rCPS in many brain regions, including thalamus, hippocampus, and parietal cortex compared to vehicle-treated Tsc2+/- mice (n=4). Rapamycin treatment did not significantly alter rCPS in controls. In vehicle-treated animals, we confirm decreased protein synthesis in Tsc2+/- mice (n=4) compared to controls (n=3). Our preliminary results suggest a complex role of mTORC1 in the regulation of cerebral protein synthesis, a role that is sensitive to rapamycin treatment.

Disclosures: **R.M. Reith:** None. **T. Huang:** None. **T. Burlin:** None. **C. Beebe Smith:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: NIMH MH103680

Wadsworth Center

Title: Congenic and BAC transgenic manipulations of BTBR mice correct interhemispheric connectivity defects

Authors: *A. SNYDER-KELLER[1,2], K. MANLEY[1], K. KLUETZMAN[1], V. J. BOLIVAR[1,2]; [1]Wadsworth Ctr., New York State Dept. of Hlth., Albany, NY; [2]Biomed. Sci., U Albany Sch. of Publ. Hlth., Albany, NY

Abstract: Investigations into the pathological basis of autism spectrum disorder (ASD) have benefitted from the study of mouse strains that model both behavioral and neuroanatomical characteristics. Inbred strains that exhibit reduced social behavior and increased repetitive behaviors, such as BTBR T+Itpr3tf/J (BTBR), also have decreased interhemispheric connectivity. Using a combination of congenic (FVB/NJ (FVB) donor DNA) and BAC transgenic (C57BL/6J (B6) source DNA) strains on a BTBR background, we have identified a locus on the distal end of Chromosome 4 that, when replaced/supplemented with DNA from a "normal" strain (FVB or B6), corrects the connectivity defect seen in BTBR mice. The corpus callosum is fully formed in the majority (over 70%) of our heterozygous congenics, and in 100% of those homozygous for FVB at this locus. The majority (60%) of BAC transgenics were also fully corrected. The remaining mice exhibited a "partial correction" phenotype that consisted of a normal corpus callosum appearance caudally, but a failure of fibers to cross in more rostral sections. Interestingly, a smaller percentage of females - both congenic and transgenic - were fully corrected compared to males. Here we present a comprehensive analysis of forebrain connectivity, using a non-suppressive silver stain for intact neural fibers (Lund & Westrum modification of the Nauta-Gygax technique). Semi-quantitative assessment of the rostral-caudal extent of crossing fibers will be related to behavioral performance in the congenic and transgenic mice. We also use DiI tracing of commissural projections in neonates and adults to demonstrate that cortical axons fail to cross in the absence of a copy of the wildtype locus, despite the presence of a truncated bridge of midline tissue in BTBR mice in place of a full corpus callosum and hippocampal commissure. In combination with immunostaining for neuronal and glial markers in perinatal brains, we are investigating the mechanisms involved in misrouting of callosal axons during development in BTBR mice. Elucidation of the genes responsible for corpus callosum defects in this mouse model of ASD may inform more complete anatomical analyses of neural connectivity defects that are at the core of developmental disorders.

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Poster

684. Developmental Disorders: Animal Models I

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Support: FCT (FOXNET)

SAFARI

Bial Foundation

ERC

Title: Foxp2 function in the adult brain

Authors: *C. A. FRENCH[1], M. CORREIA[1], S. E. FISHER[2], R. M. COSTA[1];
[1]Champalimaud Ctr. For the Unknown, Lisbon, Portugal; [2]Max Planck Inst. for Psycholinguistics, Nijmegen, Netherlands

Abstract: Disruptions of the FOXP2 gene cause a rare speech and language disorder. In the KE family a heterozygous FOXP2 mutation is dominantly inherited and affected individuals have difficulty producing the sequences of orofacial motor movements necessary for fluent speech. This is considered a core deficit of the disorder, although other expressive and receptive language problems also exist. The FOXP2 transcription factor is expressed in cortico-striatal/ -cerebellar circuits required for sensorimotor integration and motor-skill learning, and imaging studies have identified structural abnormalities in several of these regions in affected KE-family members. FOXP2 is also highly conserved in a number of other vertebrate species, where expression is seen during development and in adulthood. Mice carrying the KE-family mutation have motor-skill learning deficits and lack striatal long-term depression. They also have abnormally high striatal activity *in vivo* which is aberrantly modulated during the learning of a motor task. Juvenile zebra finches show increased FoxP2 expression during the song learning period in striatal nucleus Area X, and FoxP2 knockdown in this region results in inaccurate and incomplete song imitation. More recently, FoxP2 knockdown in Area X of mature birds was shown to render song more variable and abolished the mediation of song by social context, implicating FoxP2 in adult as well as developmental neural function. We used a conditional Foxp2 line and a tamoxifen-inducible Cre (CAGGS-CreER) to disrupt Foxp2 globally in adult mice. Tamoxifen was administered at 10 weeks of age and substantial Foxp2 deletion was seen 60 days thereafter. Around one third of Foxp2-flox/flox; CAGGS-Cre animals died, with the first

deaths occurring 6 weeks after tamoxifen administration. Surviving animals appeared healthy and their performance was indistinguishable from that of littermate controls on the accelerating rotarod. An operant lever-pressing task was used to examine motor-sequence learning in detail in the surviving group. In this task mice must complete 8 lever presses to trigger the release of a sucrose reinforcer. Initially the task is self-paced, but after 12 days of training a time constraint is added and the 8 presses must be completed at increasingly high speeds. Foxp2-flox/flox; CAGGS-Cre mice were able to learn the lever-pressing skill but rates of reinforcer delivery and lever pressing were reduced during both phases of training. Results are compared and contrasted with those from mice carrying aetiological and region-specific Foxp2 disruptions.

Disclosures: **C.A. French:** None. **M. Correia:** None. **S.E. Fisher:** None. **R.M. Costa:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: NIH Grant R00/NS076661

Title: Region-specific requirement for ERK/MAPK signaling in regulating GABAergic interneuron number and excitatory synaptic drive during development

Authors: J. S. MARTINEZ[1], J. D. NICHOLS[2,1], T. R. ANDERSON[2], *J. NEWBERN[1]; [1]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [2]Dept. of Basic Med. Sci., Univ. of Arizona - Col. of Med., Phoenix, AZ

Abstract: The precise function of ERK/MAPK signaling in distinct neuronal subtypes during normal and pathological brain development remains unclear. We conditionally inactivated ERK/MAPK in specific subsets of mouse embryonic GABAergic interneurons to define the functions of ERK/MAPK signaling in developing inhibitory interneuron circuits *in vivo*. Loss of ERK/MAPK signaling in the entire GABAergic population with VGAT/VIAAT:Cre led to neonatal growth delay and lethality by the second postnatal week. Interestingly, the total number of cortical GABAergic neurons was intact at neonatal stages. To circumvent early lethality, we employed an Nkx2.1:Cre line to delete ERK/MAPK in a restricted population of GABAergic neurons arising from the medial ganglionic eminence. Immunolabeling of mature recombined GABAergic neurons in adult mutants did not reveal significant changes in total cortical GABAergic neuron number. In striking contrast, we found a substantial decrease in somatostatin

(SST) expression in cortical GABAergic neurons as early as P14, while parvalbumin expression was relatively less effected. SST expression has been linked to changes in neuronal activity and CREB mediated transcription. Indeed, electrophysiological recordings of recombined P14 GABAergic neurons revealed a decrease in the frequency of spontaneous excitatory post-synaptic potentials in mutant sensory cortices. In contrast to the cortex, we found that loss of ERK/MAPK signaling significantly reduced the total number of GABAergic interneurons in the dentate gyrus in both VGAT/VIAAT:Cre and Nkx2.1:Cre expressing mutants. Our data reveal that in developing GABAergic circuits, ERK/MAPK is necessary for establishing appropriate number in the hippocampus, while in the cortex, ERK/MAPK signaling is required for excitatory synaptic drive and SST expression. These findings suggest that the pathogenesis of neurobehavioral changes in forms of RASopathies and schizophrenia linked to reduced ERK/MAPK signaling may involve region and subtype specific alterations in developing GABAergic circuits.

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Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: KAKENHI 25116516

KAKENHI 25350987

Title: Analysis of Gtf2i mutant mice exhibiting social behavioral abnormality

Authors: *S. UEDA[1], A. SAWA[2], T. SAKURAI[1];
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Abstract: Rare genome copy number variations (CNVs) caused by micro-deletion/duplication of chromosome play significant roles in psychiatric disorders. Altered functions of genes inside/nearby CNVs contribute to phenotypes of the disorders. Developmental neuropsychiatric syndromes associated with 7q11.23 CNV have been identified, deletion being with Williams-Beuren syndrome (WBS) that shows hypersociability, opposite to behavioral characteristics of

autism. Interestingly, 7q11.23 duplication is associated with autism, suggesting that genes affected by the CNV may be crucial for development of social neurocircuitry. One of genes in the region, GTF2I, has been implicated in the hypersociability and visuospatial deficits of WBS by genotype-phenotype correlation studies of patients with atypical deletions. To assess whether and how general transcription factor II-I (Gtf2i) is involved in the social behaviors, we established two mouse lines; Gtf2i heterozygous knockout (Gtf2i-het) mice and Gtf2i BAC transgenic (Gtf2i-TG) mice mimicking deletion and duplication, respectively. Gtf2i-het mice displayed more social exploration, reminiscent of hypersocial behavior of WBS. On the other hand, Gtf2i-TG mice showed impaired sociability and enhanced repetitive behaviors relevant to autism. These results indicate that Gtf2i plays important roles in the circuitry governing social behaviors. To identify the substrates of Gtf2i responsible for social behavior, we are analyzing gene expression by using RNA-seq on Gtf2i-het and Gtf2i-TG mice. Furthermore, to clarify the circuitry activity changes of these mice, we are currently performing whole brain activity mapping after social interaction.

Disclosures: **S. Ueda:** None. **A. Sawa:** None. **T. Sakurai:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: Telethon Grant

Title: Altered adult neurogenesis and enhanced seizure propensity in oligophrenin-1 knock-out mice, a murine model of X-linked intellectual disability

Authors: ***M. ALLEGRA**[1], C. SPALLETTI[2], B. VIGNOLI[3], S. AZZIMONDI[1], I. BUSTI[4], M. CANOSSA[3], M. CALEO[1];
[1]Inst. Di Neuroscienze CNR, Pisa, Italy; [2]Scuola Superiore Sant'Anna, Pisa, Italy; [3]Univ. di Bologna, Bologna, Italy; [4]Univ. degli Studi di Pisa, Pisa, Italy

Abstract: Oligophrenin-1 (OPHN1) gene is located on the X chromosome, whose mutations cause X-linked intellectual disability and epilepsy. OPHN1 encodes a RhoGTPase-activating protein (Rho-GAP) that regulates neuronal morphology, cell proliferation and migration, and participates in synaptic function. OPHN1 knock out (KO) mice exhibit impairments in spatial memory and social behavior, an immature phenotype of dendritic spines in CA1 pyramidal

neurons associated with altered synaptic plasticity. However, how mutations in OPHN1 affect circuit formation, with consequent cognitive impairment and network hyperexcitability remains still incompletely understood. A useful model to investigate neural network formation and function is adult hippocampal neurogenesis. Using labeling of newborn neurons with bromodeoxyuridine (BrdU) at different time point, doublecortin and retroviral vectors expressing GFP, we found significant impairments in differentiation, migration and integration of newborn neurons in the dentate gyrus of OPHN1 KO mice as compared to controls. We found an altered morphological maturation of newly generated cells, in particular a lack of axonal extension towards the CA3 area and reduced neuronal survival. Some of these pathological phenotypes could be rescued by systemic administration of fasudil, an inhibitor of the Rho kinase (ROCK) whose activity is potentially upregulated by loss of OPHN1. Moreover, since electrophysiological studies indicated an impairment of GABAergic synaptic transmission in hippocampal slices of OPHN1 KO mice, we investigated seizure propensity in KO and wild type (WT) animals. By *in vivo* recordings of hippocampal activity in freely moving animals (two months of age), we found significant epileptiform alterations in OPHN1 KO but not WT mice. We also studied seizure activity induced by administration of the glutamatergic agonist kainic acid, and we found higher susceptibility to seizures in OPHN1 KO mice. This was accompanied by neuropathological alterations such as GABAergic interneuronal death and neuropeptide Y (NPY) upregulation. Altogether, our results demonstrate that the loss of function of OPHN1 affects the adult hippocampal circuitry, causing alterations in neurogenesis and enhanced seizure susceptibility.

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Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Title: Neonatal one-day binge-like ethanol exposure results in an acute loss of neurons in the anteroventral and anterodorsal thalamic nucleus in the rat: a stereological investigation

Authors: *R. M. NAPPER, G. GIBSON, J. MITTENDORFF-GOODALL;
Univ. of Otago, Dunedin, New Zealand

Abstract: Apoptotic cell death in the developing brain has been established as a key factor in alcohol induced developmental brain damage (Idrus and Napper, 2012). The thalamic nuclei have been shown to be susceptible to the toxic effects of ethanol during development in the mouse but this effect has not been quantified or the long-term effects assessed (Wozniak et al., 2004). The present study used stereological methods to quantify the magnitude of apoptotic cell death, phenotype of dying cells and long-term neural deficit following a single binge exposure to ethanol on postnatal day 7 (PN7) in the anterodorsal (AD) and anteroventral (AV) thalamic nuclei of the rat. On PN7 Long Evans rat pups, were randomly assigned to either an alcohol exposed (AE) group, 6g/kg/day in artificial milk solution given in two feeds two hours apart or a sham intubation (SI) group. Peak BEC was 335±76 in AE animals (mean ± sd). At either 12 hours after initial alcohol exposure, or on PN60 animals were anaesthetized and perfused. The brains removed, cryo-protected, frozen and serial 50μm thick sections cut through the thalamus. The optical fractionator method was used on a systematic random subset of cresyl violet stained sections to estimate total number of apoptotic cells (12 hours) or total cell number (PN60). A subset of sections, immunostained for NeuN and counterstained with Hoescht were used to phenotype apoptotic neurons. Ethanol treatment resulted in a significant increase in the total number of apoptotic cells in the AD (E12hrs: 1991 ± 434.8; IC: 90.5 ± 32.23; SC: 12.25 ± 12.25) (mean ± SEM) ($p<0.05$) and AV (E12hrs: 20111 ± 3429; IC: 182.7 ± 41.33; SC: 113.3 ± 42.93) ($p<0.01$) thalamic nuclei. Within the AV neurons were identified in the group of apoptotic cells. This study has shown that a single binge ethanol exposure is sufficient to induce significant apoptotic cell death in the AD and AV thalamic nuclei, within 12 hours of the initiation of ethanol exposure. The long-term effect on the thalamic nuclei is under investigation. This study provides further evidence that pregnant women should abstain from binge drinking at any stage during their pregnancy, particularly during the third trimester.

Disclosures: **R.M. Napper:** None. **G. Gibson:** None. **J. Mittendorff-Goodall:** None.

Poster

684. Developmental Disorders: Animal Models I

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: NIH R01 MH104603

EU COST Action CM1103

Tourette Syndrome Association

Kansas Strategic Initiative Grant

Title: Allopregnanolone mediates tics and sensorimotor gating in the D1CT-7 animal model of Tourette syndrome

Authors: *L. J. MOSHER[1], S. C. GODAR[1], G. PINNA[2], M. BORTOLATO[1];
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Abstract: Tourette syndrome (TS) is a neurodevelopmental disorder characterized by repetitive motor and phonic tics. Tics are typically triggered by premonitory urges. These are highly intrusive feelings of hypersensitivity and discomfort, which have been posited to reflect deficits in sensorimotor gating, a core function aimed at filtering relevant information. TS patients exhibit disruptions in the prepulse inhibition of the startle reflex (PPI), an operational index of sensorimotor gating. Studies indicate that TS symptoms are underpinned by dysfunctions in dopaminergic signaling, leading to an imbalance in excitation/inhibition within cortico-striato-thalamocortical circuits. Dopamine receptor activation has been shown to elicit stereotyped behaviors and PPI deficits in rodents. The prototypical treatments for TS patients mainly consist of dopamine receptor antagonists; however, these compounds exhibit mixed effectiveness and are associated with extrapyramidal and metabolic side effects. Previous studies from our lab have investigated the therapeutic potential of targeting 5α-reductase, the main rate-limiting enzyme that catalyzes the synthesis of neurosteroids. Treatment with the 5α-reductase inhibitor finasteride (FIN) reduces tics in TS patients without the accompanying extrapyramidal symptoms, and ameliorates PPI deficits and stereotyped behaviors induced by dopamine receptor agonists in rodents. The neurosteroid(s) responsible for these actions have remained largely elusive. TS patients exhibit a hypersensitivity to stress and symptoms are exacerbated by stress. In line with these studies, we have explored the effects of a mild stress, space confinement, on the D1CT-7 mice, the only model of TS that exhibit spontaneous tic-like behaviors. We found that confinement exacerbates tic-like responses and elicits PPI deficits, both of which are attenuated by FIN. These findings led us to study the role of the 5α-reduced neurosteroid allopregnanolone (AP), a key neurosteroid in the stress cascade, on PPI deficits and tic-like behaviors in D1CT-7 mice. We found that AP did not further intensify the effects of confinement, but administration of AP to mice under normal conditions (home cage, no confinement) dose dependently increased tic-like behaviors and induced PPI deficits in D1CT-7, but not wild-type littermates. In addition, AP (15mg/kg, IP), reversed the effects of FIN (25mg/kg, IP) on PPI deficits in D1CT-7 mice. These data suggest not only that the therapeutic potential of FIN is possibly due to a reduction in AP synthesis, but also that AP may be a key neurosteroid in the mechanism whereby stress exacerbates TS symptoms.

Disclosures: L.J. Mosher: None. **S.C. Godar:** None. **G. Pinna:** None. **M. Bortolato:** None.

Poster

684. Developmental Disorders: Animal Models I

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Support: CBIR12MIG011 from NJCBIR

TS110033 from DOD-CDMRP-TSCRP

Title: Development of Pten mutant neuronal cultures as an *in vitro* model of cortical dysplasia

Authors: *I. K. NIKOLAEVA[1], P. SWIATKOWSKI[1], T. KAZDOBA[2], B. CROWELL[2], G. MAESTRI[2], B. L. FIRESTEIN[2], G. D'ARCANGELO[2];
[1]Grad. Program for Mol. Biosci., [2]Cell Biol. & Neurosci., Rutgers, the State Univ. of New Jersey, Piscataway, NJ

Abstract: Cortical dysplasia is a group of developmental brain disorders characterized by anatomical malformations of the cerebral cortex, cellular overgrowth, intellectual disability, and drug-resistant childhood epilepsy. In humans, this disorder has been associated with somatic mutations in the PI3K/Akt/mTOR signaling cascade, which is a major pathway that promotes cell growth and repair. To model the molecular abnormalities underlying cortical dysplasia, we previously generated a mutant mouse line in which the *Pten* gene is conditionally disrupted in excitatory neurons of the developing forebrain (NEX-*Pten*). Since the Pten phosphatase normally suppresses PI3K/Akt/mTOR signaling, this genetic manipulation results in the pathway's activation, specifically in these neurons. We then established forebrain neuronal cultures derived from mutant embryos as an *in vitro* model of the disorder to facilitate the identification of pharmacological treatments. We found that mutant neurons exhibited dramatic cellular hypertrophy, including increased soma size and dendrite complexity. In addition to these morphological abnormalities, Pten mutant cultures exhibited altered electrophysiological properties. We then tested compounds that are known to inhibit specific components of the PI3K/Akt/mTOR pathway to identify agents that effectively rescue the abnormalities of NEX-*Pten* mutant neurons without affecting neuronal viability. Here, we report on the beneficial effects of FDA-approved compounds, such as the Akt inhibitor MK2206 and the mTORC1 inhibitor RAD001 (a rapamycin analog). These findings will help to develop novel pharmacological treatments for children affected by cortical dysplasia. This work is supported in part by multiprogrammatic grant CBIR12MIG011 from the New Jersey Commission on Brain Injury Research (G.D. and B.L.F.), and by Exploration-Hypothesis Development Award #TS110033 from the Department of Defense - CDMRP - TSCRP (G.D.).

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Poster

684. Developmental Disorders: Animal Models I

Location: Hall A

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Autism Science Foundation Pre-doctoral Fellowship

NSF GRFP

Title: Determining the role of gtf2i family transcription factors in social behavior and oxytocin regulation

Authors: ***N. D. KOPP**, J. DOUGHERTY;
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Abstract: William's syndrome is a neurodevelopmental disorder with a prevalence of 1/7000 births. It is caused by a microdeletion of 1.8 Mbp on chromosome 7q11.23, which results in cardiovascular, craniofacial, visual-spatial, and social aberrations. Further, human studies of patients with William's syndrome have discovered that there is an increase in plasma oxytocin levels compared with typically developing controls. Two general transcription factor 2I genes, *GTF2I* and *GTF2IRD1*, are commonly deleted and are high priority candidates for mediating the hypersocial phenotype in humans and mouse models. However, it is not clear if they act independently or collaboratively to produce phenotype. We have used the CRISPR/Cas9 system to delete these transcription factors in various combinations in mice to investigate how the interaction of these genes affect the social phenotype and understand possible molecular intermediaries that manifest the hypersocial behavior. To characterize the lines, we employ a targeted sequencing technique, MDiGs, to observe large and small-scale genome editing events

within and between these genes, and perform extensive biochemical validation of the lines. Next, we have initiated longitudinal behavioral phenotyping beginning with pup ultrasonic vocalization to examine social and communication deficits in the mouse lines. Finally, to reconcile the human observation of increased oxytocin and previous mouse behavior data, we investigate the plasma oxytocin levels of *gtf2i*$^{+/-}$, *gtf2ird1*$^{+/-}$, and *gtf2i*$^{+/-}$/*gtf2ird1*$^{+/-}$ mice. We show that we have successfully knocked down *gtf2i* and *gtf2ird1* expression and report on the developmental communication behavior of ultrasonic vocalization. We show that haploinsuffiency of these two transcription factors alter the levels of oxytocin in the plasma of mice. These results suggest that oxytocin could play a salient role in the hypersocial phenotype seen in patients with William's syndrome. This observation can lead to a better understanding of the genetic contribution to the abnormal sociality seen in this syndrome and identify new mechanisms for the regulation of oxytocin.

Disclosures: **N.D. Kopp:** None. **J. Dougherty:** None.

Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: Swiss National Science Foundation 310030_146217

Title: Transgenerational transmission and modification of behavioral deficits induced by prenatal immune activation

Authors: *U. WEBER-STADLBAUER, U. MEYER;
Univ. Zurich - Vetsuisse, Zurich, Switzerland

Abstract: Objective: It has been demonstrated that environmentally induced brain dysfunctions are not solely expressed by the individuals directly exposed, but can also be transmitted to the offspring, sometimes across multiple generations. Here we explored whether such transgenerational effects can be induced by prenatal infection, an established environmental risk factor of developmental neuropsychiatric disease. To do so, we used a well-established prenatal infection murine model, which has been shown to induce long-term deficits in numerous behavioral domains in the offspring. Methods: Pregnant mice (F0) were injected with the viral mimetic poly(I:C) (5 mg/kg, iv) or control solution (saline, iv) in early pregnancy (gestation day 9), and we examined behavioral effects in direct descendants (F1). To examine whether such

effects can be transmitted to subsequent generations, we generated F2 and F3 offspring by inter-crossing F1 and F2 offspring, respectively. Results: Behavioral analyses in F1, F2 and F3 offspring revealed that deficits in social interaction and cued fear conditioning, which emerge in F1 offspring, are also present in the F2 and F3 generation. Interestingly, deficits in sensorimotor gating, observed in the F1 generation, were not present in F2 and F3 offspring. We further observed reduced amphetamine sensitivity in F2 offspring, which in turn is typically increased in F1 animals. Conclusions: Our findings demonstrate that behavioral deficits induced by prenatal infection can be transmitted and modified across subsequent generations. Future experiments will examine the possibility that the behavioral abnormalities following prenatal immune activation are transmitted to subsequent generations via modifications in the epigenetic machinery.

Disclosures: U. Weber-Stadlbauer: None. **U. Meyer:** None.

Poster

684. Developmental Disorders: Animal Models I

Location: Hall A

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Topic: C.06. Developmental Disorders

Support: Sharon Stewart Aniridia Research Trust

John and Mary Franklin Foundation

Title: Neuroanatomical abnormalities in a PAX6 deficient mouse model

Authors: *A. M. BOBILEV[1], K. K. JOHNSON[2], C. J. BLATCHER[2], K. HEKMATYAR[2], J. D. LAUDERDALE[2];
[1]Neurosci., [2]The Univ. of Georgia, Athens, GA

Abstract: The PAX6 gene encodes a highly conserved transcription factor that is expressed in the developing eye, brain, spinal cord and pancreas, and is required for various aspects of anatomical and functional development. Heterozygous loss-of-function mutations of PAX6 are causal for aniridia in humans. While the effects of PAX6 mutations on ocular development have been well characterized in human and mouse, the implications of these mutations on brain structure remain poorly understood. Previous studies have identified structural abnormalities in fiber tracts and subcortical structures in the brain including corpus callosum, anterior and posterior commissures, pineal gland and Probst bundles in persons with aniridia using magnetic

resonance imaging (MRI). The current study sought to determine whether structural brain abnormalities observed in patient case studies are present in the *Small Eye*^{Neu} (PAX6$^{Sey\ Neu/+}$) mouse model of the disorder using MRI and histological measures of brain structure. This study employed high resolution MRI using a 7T Agilent system to acquire structural brain images using both 2D T1 MEMRI (manganese enhanced MRI), T2 and 3D T2 weighted fast spin echo sequences. Sixteen mice (8 PAX6$^{Sey\ Neu/+}$, 8 wild-type littermates, age 3-4 months, outbred background) were administered $MnCl_2$ contrast agent at a rate of 40mg/kg bodyweight for 3 consecutive days prior to T1-weighted image acquisition using T1-weighted spin echo imaging (TR/TE 500/12 msec, matrix 128 x 128, nt =8 with fov of 22 x 22). The 2D T2 and 3D T2 acquisition was performed on a separate day (2D: TR/TE 4600/35 msec, nt =16; 3D: TR/TE 400/44 msec, nt=4). Both manual ROI analysis of the specific regions in MEMRI images using inbuilt analysis functions in VNMRJ and SPM VBM analyses in 2D and 3D images were performed to detect differences. Statistics were performed on a voxel -by-voxel basis to identify significant differences in volume (FDR <.05) between genotype groups. Upon completion of *in vivo* imaging, animals were euthanized following approved protocols, and their brains examined for structural abnormalities. Preliminary analyses demonstrate significant differences in brain volume in regions where PAX6 is known to be expressed during neural development. Further, preliminary histological data suggests PAX6$^{Sey\ Neu/+}$ mice have larger lateral ventricles compared to wild-type littermates. These results provide evidence for neuroanatomical abnormalities in mice with haploinsufficient levels of PAX6, presenting new avenues for investigation with regard to regionalized PAX6 necessity during brain development as well as the neuroanatomical implications of PAX6 mutations in humans.

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Poster

684. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

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Program for the Advancement of Keio Next Generation Research Projects

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Title: Neuronal heterotopias affect the activities of distant brain areas and lead to behavioral deficits

Authors: *K. ISHII[1], K.-I. KUBO[2], T. ENDO[3], K. YOSHIDA[2], S. BENNER[3], Y. ITO[4], H. AIZAWA[4], M. ARAMAKI[2], A. YAMANAKA[5], K. TANAKA[4], N. TAKATA[2], K. F. TANAKA[2], M. MIMURA[2], C. TOHYAMA[3], M. KAKEYAMA[6], K. NAKAJIMA[2];
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Abstract: Neuronal heterotopia refers to brain malformations resulting from deficits of neuronal migration. Individuals with heterotopias show a high incidence of neurological impairments, such as epilepsy. More recently, it has come to be recognized that focal heterotopias may also show a variety of psychiatric symptoms, including cognitive and behavioral deficits. However, since focal heterotopias are not always located in the brain areas responsible for the symptoms, the causal relationship between the symptoms and heterotopias remains unclear. In this study, we showed that mice with focal heterotopias in the somatosensory cortex generated by *in utero* electroporation exhibited spatial working memory deficit and low competitive dominance behavior, which have been shown to be closely related to the activity of the medial prefrontal cortex (mPFC) in rodents. Analysis of the mPFC activity revealed that the immediate-early gene expression was decreased and the local field potentials (LFPs) of the mPFC were altered in the mice with heterotopias as compared to the control mice. This difference in the LFPs between the mice with heterotopia and the control mice disappeared after optogenetic activation of the ectopic neurons and their sister neurons in the overlying cortex. Moreover, activation of these ectopic and overlying sister neurons using the DREADD (designer receptor exclusively activated by designer drug) system also improved the spatial working memory deficits. These findings suggest that cortical regions containing focal heterotopias can affect distant brain regions and give rise to behavioral abnormalities, and provide mechanistic insight into the various symptoms associated with heterotopia.

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Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NIH R00 NS076661

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Title: Hyperactivation of ERK1/2 signaling in developing GABAergic circuits reduces parvalbumin interneuron number and increases cortical excitability

Authors: *M. A. MORENO[1], L. T. HEWITT[1], G. R. BJORKLUND[1], C. W. DANIELS[2], M. F. OLIVE[2], F. SANABRIA[2], S. MARSH[3], D. M. TREIMAN[3], W. D. SNIDER[4], J. M. NEWBERN[1];
[1]Sch. of Life Sci., [2]Sch. of Psychology, Arizona State Univ., Tempe, AZ; [3]Dept. of Neurol., Barrow Neurolog. Inst., Phoenix, AZ; [4]UNC Neurosci. Ctr., Univ. of North Carolina Sch. of Med., Chapel Hill, NC

Abstract: The MAP (Mitogen-activated Protein) kinases, ERK1 and ERK2 (extracellular signal-regulated kinase) govern important aspects of nervous system development. Gain of function signaling through ERK1/2 is commonly observed in the Rasopathies, aka Ras/MAPK syndromes or NCFC syndromes, a family of syndromes with deficits in cardiac, cranial, ectodermal, and nervous system development. Previous studies have suggested that mature GABAergic inhibitory interneuron function is disrupted in Ras/MAPK syndromes. However, it remains unknown whether alterations in the initial development of GABAergic circuits is an important feature of disease pathogenesis. We investigated the effects of ERK1/2 signaling hyperactivation on the early development of cortical GABAergic interneurons by crossing VGAT:Cre expressing mice with a Cre-dependent constitutively activated MEK1S217/2221Q allele. Our results show that hyperactivation of ERK1/2 signaling in inhibitory interneurons during embryogenesis leads to a significant decrease in the total number of neocortical GABAergic interneurons. Interestingly, this decrease is primarily confined to a specific subset of inhibitory neurons that expresses parvalbumin. The reduction in the number of parvalbumin expressing neurons is detectable by P14 in sensory cortices. RNA immunoprecipitation and sequencing of cortical GABAergic neuron-specific mRNA identified broad changes in the gene expression profile during neonatal stages that indicate accelerated lineage progression in mutant mice. To better

understand the behavioral consequences of ERK1/2 hyperactivation in GABAergic circuits, mice were tested in the elevated plus maze, open field test, social approach task, and a fixed interval schedule of reinforcement. Though no significant differences were observed in these neurobehavioral tests in mutants, we detected a striking increase in freezing behavior during open field testing. Direct electroencephalography (EEG) recordings provided evidence of enhanced epileptiform-like brain activity in mutant mice. These data suggest that hyperactivation of ERK1/2 accelerates the trajectory of GABAergic neuron development, leading to a postnatal reduction in parvalbumin interneuron number and seizure-like activity in the neocortex. Defining the mechanisms regulated by ERK1/2 in GABAergic circuits may assist in our understanding of the pathogenesis of epilepsy and E/I imbalances in Ras/MAPK Syndromes.

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Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: R01-MH074736

R37-MH065635

T32-MH019524

Title: Mechanisms by which systemic administration of insulin-like growth factor ii reverses autism spectrum disorder phenotypes in mice

Authors: ***A. B. STEINMETZ**, S. A. STERN, A. S. KOHTZ, C. M. ALBERINI;
New York Univ., New York, NY

Abstract: Autism spectrum disorder (ASD) is a developmental disability characterized by altered social interaction and cognitive functions as well as increased repetitive behavior. Thus far, there is no efficacious therapy that can ameliorate most of the major ASD symptomatology, and the identification of potential novel targets and therapies is urgently needed. We used BTBR mice, a behaviorally well-characterized mouse model that reproduces behaviors typical of ASD, to test the effects of systemic administrations of insulin like growth factor II (IGF-II). Previous

results from our lab have shown that intrahippocampal or systemic administration of IGF-II in rats or mice results in enhanced short and long term memories in a variety of paradigms. Here we report that IGF-II rescues the three major behavioral deficits of ASD modeled in the BTBR mice. Specifically, IGF-II increased social interactions and memory, contextual memory, and decreased repetitive behaviors. The treatment did not affect locomotor or anxiety-like behaviors. Blocking the IGF-II but not the IGF-1 receptor within the hippocampus prevented the IGF-II rescuing of social and contextual memory. Furthermore, an increase of the AMP-activated protein kinase (AMPk)- mammalian target of rapamycin (mTOR) - p70 S6 kinase (S6k) pathway in BTBR synaptoneurosomal fractions as compared to control animals was found. The administration of systemic IGF-II along with contextual fear conditioning reduced the levels of AMPk, mTOR, and S6k to control levels. Thus, IGF-II treatment seems to act via regulation of the AMPk-mTOR-S6k pathway to rescue ASD phenotypes. Funding: R01-MH074736 and R37-MH065635 to C.M.A. and T32 MH019524 to A.B.S.

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Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

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Title: Deficiency and haploinsufficiency of bridging integrator 1 (Bin1) causes abnormalities in the developing mouse brain

Authors: *K. D. ONOS[1], L. C. GRAHAM[1,2], C. J. ACKLIN[1], X. WANG[1], G. W. CARTER[1], G. R. HOWELL[1,2];
[1]The Jackson Lab., Bar Harbor, ME; [2]Tufts Univ., Boston, MA

Abstract: *Bin1* (also known as *Amphiphysin II*) belongs to the BAR (Bin/Amphyiphysin/Rvs) family of genes that support a diverse number of cellular processes such as endocytosis, endosome trafficking, actin dynamics, calcium signaling and stress signaling through neuroendocrine cells (Sarret et al., 2004). Variations in *Bin1* have been associated with Alzheimer's disease, autism and schizophrenia (Yang, et. al., 2008; Butler, et. al, 2015;Chauhan et. al, 2015) but the mechanisms are not known. *Bin1* has been shown to play a critical role in

development as homozygous knockout mice exhibit perinatal lethality due to severe ventricular cardiomyopathy (Muller et al., 2003), and this has impeded further work to fully understand the role of *Bin1* in the developing brain. Therefore, we are using genetic and genomic approaches using *Bin1* homozygote (*Bin1* deficient), heterozygote (haploinsufficient) and conditional knockout mice to elucidate the role of *Bin1* in the developing mouse brain. To determine the impact of *Bin1* deficiency on brain development we first examined brains of *Bin1* sufficient, deficient and haploinsufficient mice for structural and molecular changes at P0 (all genotypes) and P60 (sufficient and haploinsufficient only). Although there were no gross morphological changes between genotypes, using antibodies to PSD95, NEUN, phosphorylated neurofilament and myelin basic protein, differences were observed between genotypes to both synapses and axons of neurons in the amygdala, CA1 and CA3 regions of the hippocampus and the dentate gyrus. Secondly, we have performed gene profiling by RNA-seq on brains from *Bin1* haploinsufficient mice at 6 months of age compared to *Bin1* sufficient controls. A total of 221 genes were upregulated and a 117 genes downregulated (338 in total), including a 1.5 fold downregulation of *Bin1*. Gene set enrichment analysis identified genes associated with membrane-bounded organelles, in particular localized to the mitochondria, as enriched in this dataset. Finally, to fully understand the role of *Bin1* in development and aging we have obtained a floxed allele of *Bin1* and are employing a Cre/LoxP strategy to generate mice deficient in *Bin1* in neural progenitors. Taken together, these findings show that *Bin1* deficiency and haploinsufficiency impact critical process in the developing brain and highlight the importance of determining the mechanism(s) by which *Bin1* contributes to normal brain function and disease.

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Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NSERC of Canada

AIHS

Title: The effects of *in utero* valproic acid exposure on placental transfer and brain development in autism: A T2 MR study

Authors: *L. S. TRUICA[1], S. RAZA[2], J. K. MCCREARY[2], I. Q. WHISHAW[2], R. GIBB[2]; [1]Neurosci., Canadian Ctr. For Behavioral Neurosci., Lethbridge, AB, Canada; [2]Canadian Ctr. for Behavioural Neurosci., Lethbridge, AB, Canada

Abstract: Neurobiological anomalies have been reported in autism spectrum disorders (ASD), although the mechanisms underlying these changes are not well known. Human neuroimaging studies have shown abnormal regulation of brain growth in ASD, where processes governing apoptosis and synaptic pruning are highly implicated. The aberrant cortical connectivity observed in ASD has been hypothesised to be correlated to accelerated early neuronal growth, followed by excessive synaptic pruning. The present study investigated brain tissue changes in the valproic acid (VPA) animal model of ASD using MRI, as well as placental transfer of the drug. Pregnant dams were exposed orally to VPA on gestational day (GD) 12.5. Brain and placental *in vivo* T2-relaxometry measurements were conducted on GD 16, 18 and 20 using a 4.7 T Oxford magnet (Oxford, UK). On postnatal (P) day 30, P35, P40, and P45, the offspring were also imaged. Parametric maps were constructed for both rat placentas and the pup brains. The offspring were also behaviourally tested in the pre-weaning period to assess changes in emotional regulation and sensorimotor development, two predominant symptoms of autism. MRI results showed differences between VPA and control groups in the prefrontal cortex, suggesting that prenatal exposure to VPA altered the expected neuronal density. This may reflect a delay in synaptic pruning, which is consistent with human ASD literature. The drug placental transfer was also evident in the T2 maps across gestational age, supporting the pharmacokinetics of the VPA in the investigated animal model. Alterations in early behavior were also exhibited, suggesting perturbations in the neurodevelopmental trajectories. Altogether, results from this study conclude that VPA impacts the offspring by crossing the placental barrier, and thereby influences neurodevelopment in early and later life. Moreover, this study indicates that T2 relaxometry is a valuable tool in detecting early life developmental abnormalities.

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Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NSERC Canada A7077

Title: Maternal influences on pup ultrasonic vocalizations in D2 and M5 deficient mice

Authors: *J. S. YEOMANS[1], D. I. WASSERMAN[3], T. CURRY[4], B. J. PEREIRA[2], P. TSELICHTCHEV[2], M. LAM[1];
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Abstract: Ultrasonic vocalizations (USVs) occur in 3-11 day old mouse pups isolated from their dams. Fewer USVs occur in pups deficient in genes for D2 dopamine or M5 muscarinic receptors, or the Gtf2i gene deficient in Williams' syndrome. We crossed heterozygous mice to quantify pup gene contributions, and made reciprocal crosses to quantify parental gene contributions to 8 day old pup USVs. In D2 deficient mice, both pups' and dams' genotype were important, with dams' genotype affecting the onset and pups' genotype affecting the gradual increase, of USVs over 4 minutes. Heterozygous D2 pup USVs increased plasma prolactin levels and maternal behaviors (nest building and pup retrieval) in wild-type, but not D2 knockout, dams. This suggests that USV activation of maternal prolactin is important for maternal behaviors in D2 mice. In M5 deficient mice, dams' genotypes were most important for pups' USVs, possibly due to M5 receptor expression in the medial hypothalamus of female mice. The roles of prolactin, oxytocin, endorphins, and other hormones associated with bonding, social and brain development, and autism are discussed.

Disclosures: J.S. Yeomans: None. **D.I. Wasserman:** None. **T. Curry:** None. **B.J. Pereira:** None. **P. Tselichtchev:** None. **M. Lam:** None.

Poster

685. Developmental Disorders: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 685.06/H39

Topic: C.06. Developmental Disorders

Title: Can a poor sleep/wake cycle contribute to hippocampal malfunction in a mouse model of neurodevelopmental disabilities?

Authors: D. H. LOH[1], H.-B. WANG[1], F. Y. LEE[2], D. S. WHITTAKER[3], K. Y. CHENG[4], E. C. DELL'ANGELICA[2], C. S. COLWELL[1], *C. A. GHIANI[5,6];
[1]Psychiatry, [2]Human Genet., [3]MCIP Program, [4]Neurosci. IDP, [5]IDDRC, Semel Inst., [6]Pathology and Psychiatry, UCLA, Los Angeles, CA

Abstract: Major psychiatric diseases with a neurodevelopmental origin are triggered by a combination of genetic predisposition and environmental stressors. It has been proposed that exogenously disrupting the circadian system may account for increased susceptibility to neuropsychiatric symptoms. Hence, we have become interested in the possibility that circadian disruption may be a trigger that can push a vulnerable patient into disease state. To test this hypothesis, we have been using the pallid mouse, which carries a mutation in the gene that encodes pallidin (bloc1s6), a subunit of the dysbindin-containing complex BLOC1 (Biogenesis of Lysosome-related Organelles Complex 1). This complex is developmentally regulated and implicated in intracellular protein trafficking. Here, we show that lack of BLOC-1 altered gene expression in the developing central nervous system (CNS) of mutant mice, eliciting defective neural cell maturation and maldevelopment of relevant brain areas, such as the hippocampus. BLOC1-deficient mice exhibited reduced levels of activity, and their activity rhythms were significantly more fragmented and of lower precision than in age-matched WT. Whilst the pallid mutants slept for the same amount of time as WT, the quality of sleep was compromised by the highly fragmented nature of their sleeping pattern. In agreement with these results, the levels of Period2, a protein involved in the regulation of the intrinsic clock were altered in the pallid hippocampus. Our data suggest that lack of BLOC-1 negatively influences CNS development and functions, perhaps leading to faulty brain wiring, as well as circadian disruption.

Disclosures: **D.H. Loh:** None. **H. Wang:** None. **F.Y. Lee:** None. **D.S. Whittaker:** None. **K.Y. Cheng:** None. **E.C. Dell'Angelica:** None. **C.S. Colwell:** None. **C.A. Ghiani:** None.

Poster

685. Developmental Disorders: Animal Models II

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Program#/Poster#: 685.07/H40

Topic: C.06. Developmental Disorders

Support: NIH 5K12GM000708-14

Title: Spontaneous motor behavior of *Drosophila* fragile X mental retardation 1 (dfmr1) mutants reveals perseverative and excessive grooming

Authors: *D. R. ANDREW[1,4,2,3], M. E. MOE[2,3], R. L. DOSER[3,2], L. L. RESTIFO[3,2];
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Abstract: Fragile X Syndrome (FXS), caused by loss of function mutations in the *fragile X mental retardation 1* gene (*FMR1*), is the leading monogenic cause of human intellectual disability (ID). In addition to the hallmark feature of ID, patients with FXS often manifest concomitant neurological disorders such as epilepsy, sleep disorders, anxiety, hyperactivity, attention deficits, autism, and decreased motor coordination. The fruit fly, *Drosophila melanogaster*, provides a well-established genetic model for FXS. Flies with null mutations in *dfmr1*, the sole ortholog of *FMR1*, show neuroanatomical and behavioral deficits, several of which resemble those in patients with FXS. Here, we present a quantitative analysis of spontaneous motor behaviors in *dfmr1* mutant flies toward the goal of a more nuanced understanding of behavioral perturbations caused by absence of dFMRP. We recorded and observed socially isolated, 1-day-old flies during a 15-minute observational period in a small circular arena and scored walking, standing, grooming, and falls. We found that the grooming index, the proportion of time spent grooming, was significantly increased in *dfmr1* mutants, and that the introduction of a wild-type $dfmr1^{+}$ transgene in a mutant background rescued this difference. We could attribute this excessive grooming to increases in both the number and mean duration of grooming bouts. We also found that mutants groomed significantly more body parts per grooming bout and showed a greater variety of grooming-site transitions compared to genetic controls. Mutant animals performed different patterns of grooming, with a marked increase in rear-body grooming transitions carried out by the middle and rear legs. We quantified repetitive grooming via two distinct pattern measures and found that mutants showed elevated numbers of these repetitions. Preliminary data indicate that the grooming phenotype is exacerbated by higher concentrations of glutamate in the food of developing larvae. Together, these attributes of excessive repetitive grooming in *dfmr1*-mutant flies are reminiscent of the perseverative behaviors observed in patients with FXS. These observations call into question the interpretation of other *dfmr1* behavioral phenotypes, notably “reduced courtship interest.”

Disclosures: **D.R. Andrew:** None. **M.E. Moe:** None. **R.L. Doser:** None. **L.L. Restifo:** None.

Poster

685. Developmental Disorders: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 685.08/H41

Topic: C.06. Developmental Disorders

Title: The impact of maternal diet on large scale network patterns and behavior in macaque offspring

Authors: *J. S. RAMIREZ[1], E. L. SULLIVAN[2], B. D. MILLS[1], J. VALLEAU[2], E. EARL[1], O. MIRANDA-DOMINGUEZ[1], D. A. FAIR[1];
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Abstract: Resting state functional connectivity MRI (rs-fcMRI) is a tool used to investigate neurophysiology *in vivo*. This tool has identified unique connectivity in an array of different populations, as well as animal models. The current project aims to investigate rs-fcMRI in macaques in order to establish developmental associations in connectivity of offspring that occur secondary to a maternal high-fat diet (HFD) during pregnancy. Maternal HFD has been associated with complex neurodevelopmental disorders such as ADHD and Autism. Thus, the goal of this project is to examine how maternal diet affects large scale network patterns, as measured via rs-fcMRI, in the macaque previously shown as atypical in these populations (I.e. Nucleus accumbens (NAc) and amygdalae connectivity). Preliminary results suggest atypical connectivity with the NAc in offspring exposed to maternal HFD, which was associated with anxiety in female offspring, repetitive behaviors in male offspring and impairments in social behavior in both male and female offspring- component dimensions of both ADHD and Autism.

Disclosures: J.S. Ramirez: None. **E.L. Sullivan:** None. **B.D. Mills:** None. **J. Valleau:** None. **E. Earl:** None. **O. Miranda-Dominguez:** None. **D.A. Fair:** None.

Poster

685. Developmental Disorders: Animal Models II

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Program#/Poster#: 685.09/H42

Topic: C.06. Developmental Disorders

Support: NIH/NCRR 8UL1TR000077

Title: The effects of creatine loss in adult creatine transporter conditional knockout mice

Authors: *K. C. UDOBI, M. R. SKELTON;
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Abstract: Creatine (Cr) transporter (CrT) deficiency (CTD) is a leading cause of X-linked intellectual disability. CTD is characterized by a lack of brain Cr, severe intellectual disability and aphasia. We generated conditional CrT knockout mice ($CrT^{flox/flox}$). Ubiquitous CrT knockout mice ($CrT^{-/y}$) and brain specific knockout mice (bKO) generated from this line exhibit

cognitive deficits, mimicking the human disorder. While it is clear that Cr is essential for proper neurologic function, it is still unknown if Cr is required for proper brain development. A better understanding of the role of Cr in brain development is essential for the development of treatments and treatment strategies for CTD. The purpose of this study is to determine if CTD is a developmental disorder or if the loss of Cr in itself is sufficient to induce learning deficits. To better understand if the cognitive deficits in $CrT^{-/y}$ mice are due to developmental changes in the brain, mice lacking the CrT only during adulthood were generated. $CrT^{flox/flox}$ mice were crossed with mice expressing a ubiquitous tamoxifen-inducible Cre recombinase (UBC-cre/ERT2) to create a temporally mediated CrT knockout mouse (cKO). Recombination was initiated by tamoxifen administration from postnatal day 60-65. Following the induction of Cre recombinase activity, cKO mice exhibit a significant reduction in body weight compared to wildtype (WT) mice. Brain CrT transcripts were undetectable 5 days following Cre recombinase induction. Following validation of the knockout, cognitive function was assessed based on deficits observed in the bKO and $CrT^{-/y}$ mice. In the visible platform testing, cKO mice show no significant difference in latency to the platform compared with WT mice. The cKO mice showed no significant difference in latency and path length during the acquisition and reversal phases of the Morris Water Maze (MWM). No reference memory deficits were present in both phases of testing. WT and cKO mice performed similarly in tasks of object recognition and fear memory. The absence of learning and memory deficits observed in the adult cKO mice suggest that the deficits seen in bKO and $CrT^{-/y}$ mice are likely due to a lack of Cr during brain development.

Disclosures: K.C. Udobi: None. **M.R. Skelton:** None.

Poster

685. Developmental Disorders: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 685.10/H43

Topic: C.06. Developmental Disorders

Title: Examination of cortizol receptor and endogenous opioid expression in a primate model of self injurious behavior

Authors: *B. FORET[1], M. JACKSON[1], J. A. FONTENOT[2], E. C. ROMERO[2], J. A. SMITH[2], D. L. HASSELSCHWERT[2], K. M. SMITH[1];
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Abstract: Self Injurious Behavior (SIB) in humans is characterized by deliberate self-harm or mutilation outside the context of societal rituals or body modification practices, and may include

head banging, self biting, self cutting, picking at wounds, or intentional burns. SIB is expressed in various neuropsychiatric disorders, most prominently in Borderline Personality Disorder, Obsessive Compulsive Disorder, Tourette Syndrome, and Autism Spectrum Disorders. SIB can lead to suicidal ideations in adolescents and adults. SIB in captive Rhesus macaques is a spontaneously emerging animal model of self-injury in humans that may aid our understanding of the neurobiology of SIB. In both humans and monkeys, SIB can be triggered or exacerbated by stressful events. Given the recurring nature of the behaviors, a reduction of endogenous opioid signaling in affected individuals (and animals) has been hypothesized based on the known release of opioids by injury, the escalating nature of self-harm, the endorsement of addictive compulsions to self injure, the high rates of substance abuse in individuals with BPD, and the effectiveness of opioid blockers in reducing symptoms of self-injury in human and animal trials. We hypothesize that the expression of stress hormone receptor and endogenous opioid signaling genes may be altered in monkeys with SIB. However, the neurobiology of these changes is poorly understood. We will examine prefrontal cortex (orbital frontal), anterior cingulate cortex, hippocampus, amygdala, and caudate putamen and hypothalamus of control and SIB animals. We have initiated these studies by collecting brain tissue form 8 rhesus macaques with SIB (4 males and 4 females) and 8 age and sex matched controls. No differences in brain weight, or brain/body weight ratio were observed. Glucocorticoid (GR) and mineralocorticoid receptor (MR) mRNA levels in the hippocampus are currently being examined. No differences in GR levels in the hippocampus of male SIB (N=3) and control males (N=3) were observed. Preliminary evidence indicates that SIB animals may have lower expression levels of MR, but additional animals need to be examined. Future studies will extend these analyses to additional brain regions for GR and MR expression, as well as genes involved in endogenous opioid signaling.

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Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: Jacobs Foundation

Fritz Thyssen Foundation

Title: Involvement of Cadherin-13 in the regulation of the response to early-life stress and environmental enrichment

Authors: *O. RIVERO, S. POPP, D. KISER, S. SICH, K.-P. LESCH;
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Abstract: Many evidences support the role of genetic risk factors for psychiatric disorders in the response to environmental changes. Genome-wide screening approaches have consistently identified cadherin-13 (*CDH13*), an atypical member of the cadherin family of cell adhesion molecules, as a risk gene for various psychiatric disorders including ADHD and autism spectrum disorders as well as substance use/dependence. The differential expression pattern of cadherin-13 within the brain suggests a functional role in different aspects of brain connectivity and plasticity (neurite outgrowth, axonal pathfinding and synapse formation). In this study, we aimed at understanding the role of Cadherin-13 in Gene x Environment interactions. For this purpose, we used a constitutive *Cdh13* knockout mouse to study the behavioral and molecular consequences of cadherin-13 deficiency in the response to early life stress (maternal separation from postnatal day P1 to P14) or environmental enrichment in early adulthood (voluntary exercise with running wheels). Our results show that maternal separation reduces anxiety-like behavior in wildtype male animals when tested in the light-dark box, whereas this effect is not observed in knockout ($Cdh13^{-/-}$) mice. This finding suggests that knockout animals respond less to MS. Furthermore, maternally separated $Cdh13^{-/-}$ animals also presented cognitive alterations during the performance of the Novel Object Recognition task and the Barnes maze. Further studies are being performed in order to elucidate the molecular mechanisms behind these behavioral alterations. On the other hand, voluntary exercise with running wheels (RW) induces, as expected, increased adult neurogenesis in all mice when compared to control, sedentary animals, although the distribution of newborn cells in the dentate gyrus (DG) suggests that $Cdh13^{-/-}$ mice are more sensitive to the effects of RW than wildtype animals, probably due to the involvement of Cadherin-13 in the migration of newborn neurons from the subgranular zone of the DG. In addition, at the behavioral level, voluntary exercise with RW induces, regardless of genotype, hyperactivity in a novel non-aversive environment, as well as impulsivity and attentional deficits in the 5 Choice Serial Reaction Time Task (5CSRTT), although the effects of GxE interactions are milder than under early-life stress conditions. In conclusion, our results support the view that cadherin-13 contributes to how the brain responds to different environmental experiences, likely via its role in structural and synaptic plasticity.

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Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

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Shriners Hospitals for Children

Title: Wdfy3 links defective cortical neurogenesis to autism spectrum disorders

Authors: *K. ZARBALIS[1], L. OROSCO[2], A. ROSS[2], S. CATES[2], S. SCOTT[2], D. WU[2], J. SOHN[2], D. PLEASURE[2], S. PLEASURE[3], I. ADAMOPOULOS[2];
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Abstract: Autism spectrum disorders (ASDs) are complex and heterogeneous developmental disabilities affecting an ever-increasing number of children worldwide. The complexity of manifestations, etiologies, and comorbidities of ASDs pose major challenges to the identification of consistent neuropathological features. Nonetheless, in a subset of young children on the autism spectrum abnormal cerebral growth trajectories have been observed. Normal cerebral size at birth is followed by a period of excessive growth, apparently the consequence of increased neuron generation. Intriguingly, we observe a reminiscent pattern of regional overgrowth in mouse models carrying deleterious alleles of the Wdfy3 gene, recently recognized as causative in ASDs. In addition, Wdfy3 mutants display focal migration defects of cortical projection neurons, a recognized cause of epilepsy, which is highly comorbid with autism. Our analysis of affected mutants identified an important function for Wdfy3 in regulating neural progenitor proliferation and neural migration in the developing brain. Specifically, Wdfy3 governs the mitosis of radial glia cells by setting the balance between proliferative and differentiative mitoses. Loss-of-function leads to an increase in proliferative divisions, an expansion of the progenitor pool, more neurons, and a larger cerebrum. In summary, our study defines an essential role for the autism factor Wdfy3 in regulating the developmental expansion and functional organization of the cerebral cortex while its loss-of-function results in pathological changes characteristic of ASDs.

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Poster

685. Developmental Disorders: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 685.13/H46

Topic: C.06. Developmental Disorders

Support: NS083513

Title: Developing a ferret model of cortical dysmaturation following newborn brain injury

Authors: *F. F. GONZALEZ[1], A. MIKHAILOVA[2], P. S. MCQUILLEN[2], S. F. SORRELLS[3], J. K. ELLIS[4];
[1]Pediatrics, UCSF, San Francisco, CA; [2]Pediatrics, [3]Neurolog. Surgery, [4]Regeneration Med., Univ. of California, San Francisco, San Francisco, CA

Abstract: Existing small animal models fail to reproduce permanent myelination failure that is the hallmark of brain injury in the preterm human newborn. Furthermore, it is unclear how closely these models recapitulate human developmental pathways because rodents have a lisencephalic cortex. Despite being a small animal (0.5-1kg), ferrets have gyrencephalic cortex with radial cortical organization and a well-developed outer subventricular zone. We aim to develop an improved translational small animal model that reproduces cerebral cortical dysmaturation that may contribute to adverse neurodevelopment following human newborn brain injury. Our specific focus is glial development in the outer subventricular zone and late cortical migratory pathways of neuronal precursors. Exposure to 10% oxygen from P10-20 in ferret has been shown to impair oligodendrocyte maturation and stimulate gliosis (Tao et al Pediatric Research (2012) 71, 192-198). We find that ten days exposure to 10% oxygen is well tolerated by the jill (N=2) and kits (N=3), with no mortality and no observable distress or morbidity. Maternal hematocrit increases (38 - 54%) during this relatively brief hypoxia exposure. Using these conditions for the model we are analyzing oligodendrocyte and astrocyte lineage progression in the outer subventricular zone and novel postnatal migratory streams of neuronal precursors using cell type specific markers and immunofluorescence. A Ferret model of human preterm brain injury may provide insight into important cerebral cortical developmental pathways that are perturbed with preterm birth, birth asphyxia and congenital heart disease in human newborns.

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Poster

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Topic: C.06. Developmental Disorders

Support: Silvio O. Conte Center for Oxytocin and Social Cognition

Title: Sex specific deficits in affective behavior in a rodent model of autism

Authors: *T. M. HENNESSEY[1], C. BARRETT[2], S. RYAN[2], L. WANG[1], D. RAINNIE[3]; [1]Neurosci., [2]Div. of Behavioral Neurosci. and Psychiatric Disorders, [3]Dept. of Psychiatry, Emory Univ., Atlanta, GA

Abstract: Development of a valid animal model of autism is a critical step in studying autism spectrum disorder (ASD). Valproic acid (VPA) exposure during the first trimester increases the risk of autism by 10 fold. By exposing developing rat embryos to VPA we can mimic the human condition with ethological and predictive validity. VPA exposed rats exhibit less social drive and increased anxiety-like behavior, as in ASD. However previous studies used intraperitoneal (IP) administration which has a high rate of fetal reabsorption. Here, we used gavage VPA administration, which markedly reduced reabsorption and is more clinically relevant. Our first aim was to recapitulate the phenotype seen when VPA is given IP. As autistic individuals are more stress sensitive, we then investigated the impact of unpredictable shock stress (USS) on social interaction and anxiety-like behavior in VPA rats. Finally, we used RNAseq to analyze gene expression changes in the basolateral amygdala (BLA) of VPA rats. Behavior was assessed in 2 pups per sex from each of 41 litters. Social drive was measured through tests of ultrasonic vocalizations (USVs), approach to maternal bedding, social preference (SP), and social novelty (SN). Anxiety-like behavior was measured in the open field (OF) and acoustic startle (AS) tests. After USS (4 days), we measured contextual freezing and OF, SP, SN and AS were retested. Notably, the effects of VPA on social interaction and anxiety were sex dependent. At P11 VPA exposed males produced significantly fewer USVs than control pups ($p=.002$), and fewer approached maternal bedding ($p=.041$). VPA exposed males also spent less time with the novel rat ($p=.033$) and more time isolated from any rat ($p=.015$) during SN. Conversely, VPA females spent less time in the center in the OF test ($p=.031$) but males showed no difference. After USS, only VPA exposed females tended to higher AS ($p=.055$). USS also tended to cause VPA males to spend less time in social context ($p=.097$). Parallel to this, the BLA of P14 and P21 rats were micropunched and sent to the Emory Genomics Core for RNAseq. There were substantial differences in genes expressed in the BLA particularly in peptide signaling, behavior, and fear

learning pathways. In sum, gavage administration of VPA to dams recapitulates the social and emotional deficits in pups seen after IP administration. We also saw sex-specific behavioral effects of VPA exposure that were exacerbated by USS. Finally, VPA exposure caused many changes in gene expression that may prove useful for finding new treatment targets to reduce anxiety and improve social function in ASD.

Disclosures: **T.M. Hennessey:** None. **C. Barrett:** None. **S. Ryan:** None. **L. Wang:** None. **D. Rainnie:** None.

Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: 4R00AA021760-02

Title: Gestational ethanol exposure during the first trimester most dramatically decreases gaba activity in the dorsolateral striatum of mice

Authors: ***S. I. SHIN**[1], V. C. CUZON-CARLSON[2];
[2]Neurosci., [1]OHSU/ONPRC, Beaverton, OR

Abstract: Alcohol is the leading preventable cause of birth defects and developmental disabilities in the US. Fetal alcohol spectrum disorders (FASD) is an umbrella term that encompasses physical, mental, and behavioral deficits. Behavioral abnormalities such as impaired motor skills, decision-making, and learning suggest alterations in cortico-basal circuitry. Within the basal ganglia, the dorsal striatum has been heavily implicated in the behavioral changes involved with addictive substances, including alcohol. We have shown that gestational exposure to ethanol (GEE) throughout a time period in mice that mimics all three trimesters of human development leads to deficits in habit formation. In the dorsolateral striatum (DLS), GEE causes an increase in median spiny neuron (MSN) firing. The increase is possibly mediated by decreased GABA input to MSNs in the DLS, as observed through a decrease in mIPSC frequency and amplitude in DLS MSNs. This decrease in GABA activity may be associated to a complementary decrease in cannabinoid receptor type 1 (CB1) activity. However, these changes were found in mice exposed to ethanol from conception through the first postnatal week, mimicking the three trimesters of human pregnancy. As a majority of alcohol consumption during pregnancy occurs within the first trimester, the aim of this study was to determine if there

is a critical period of exposure responsible for the GEE effects in the DLS. Thus, using whole cell patch clamp electrophysiology we measured the changes in DLS activity in mice that were exposed to various lengths of ethanol during pregnancy. Mice were placed in ethanol vapor chambers to produce blood ethanol levels of approximately 84 mg/dl in dams and 74 mg/dl in new born pups. Mice in the presence of ethanol during only the first trimester (to embryonic day (e)9.5) showed the most dramatic decrease in mIPSC activity in the DLS compared to control animals. Exposures up to the second (to e21) and third trimester (to postnatal day (p)10) exhibited a less profound decrease. These findings provide evidence for a critical period during gestation in which the developmental disabilities of FASD may most aggressively be inflicted. Furthermore, to substantiate our findings of a critical period of gestational ethanol exposure, we will use whole cell patch clamp electrophysiology to study CB1 receptor activity and will also look to see if these changes translate to habit formation behavior through using an instrumental learning task that can delineate goal-directed and habitual actions.

Disclosures: S.I. Shin: None. **V.C. Cuzon-Carlson:** None.

Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NIH Grant NS088766

NIH Grant TR000124

Title: Behavioral deficits and cholinergic pathway abnormalities in male Sanfilippo B mice

Authors: *P. DICKSON[1], S. Q. LE[1], S.-H. KAN[1], B. BENEDICT[1], Q. BUI[1], J. CUSHMAN[2], M. S. SANDS[3];
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Abstract: Sanfilippo syndrome causes progressive neurological deterioration and reduced fear associated with volume loss in the amygdala. We studied 12 male Sanfilippo B mice and 12 littermate controls age 16-20 weeks to determine whether this model would be similarly affected. Affected mice showed reduced anxiety, with a decrease in the number of stretch-attend postures during the elevated plus maze ($p=0.01$, $F=13.7$), an increased tendency to approach a novel

mouse ($p=0.067$, $F=3.66$), and an increased tendency to linger in the center of an open field ($p=0.032$, $F=5.28$). Radial arm maze testing showed a longer latency to find reward during training ($p=0.025$, $F=5.96$). We stained sections of 2 affected and 2 control female mice aged 41-42 weeks for acetylcholinesterase (AChE) activity for stereology to measure volume of the amygdala. Upon staining, we noticed an overall reduction in AChE activity staining in affected animals. To verify our observation, we measured AChE and choline acetyltransferase (ChAT) activity via colorometric assays in whole brain homogenates of the mice that were used in the behavioral experiment. We found a 12.4% reduction in mean AChE activity ($p<0.001$) and no difference in ChAT activity in affected animals. However, as the amygdala may be specifically affected in Sanfilippo disease, we evaluated AChE and ChAT in 3 affected mice and 3 controls using a hemicoronal section (thickness 2 mm) that contained amygdala. AChE activity was 25% lower ($p=0.006$) and ChAT activity 14% lower ($p=0.002$) in affected animals in that section of the brain. The levels of AChE and ChAT in whole brain homogenates did not show correlation with the behavioral variables that showed intergroup differences in the mice. Cholinergic pathways are affected in adult-onset dementias, including Alzheimer disease. Our results suggest that male Sanfilippo B mice display neurobehavioral deficits at a relatively early age, and that as in adult dementias, they may display deficits in cholinergic pathways.

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Poster

685. Developmental Disorders: Animal Models II

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Program#/Poster#: 685.17/I2

Topic: C.06. Developmental Disorders

Title: Too much, too early: effects of sensory overstimulation in early life

Authors: ***S. RAVINDER**[1], J. S. B. RAMIREZ[2], D. CHRISTAKIS[1], J. M. RAMIREZ[1], S. FERGUSON[1];

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Abstract: Attention deficit hyperactivity disorder (ADHD) is a common childhood neurodevelopmental disorder that has profound effects on individuals, their families and society. Most research has focused on understanding genetic causes, but there remains a large role for environmental factors in the etiology of this disease. In particular, early life experiences exert long-lasting effects on neural function, which can have a deep impact on behavior and vulnerability to developing neuropsychiatric illnesses such as ADHD. We have developed a rodent model of "excessive non-normative stimulation (ENS)" whereby mice are exposed to audio-visual stimuli for six hours per day, from P10-P52 (Christakis et al., Sci Rep., 2012). When subsequently tested, ENS mice demonstrate numerous behavioral outcomes that are reminiscent of the 3 core clinical dimensions of ADHD (inattentiveness, impulsivity, hyperactivity). Compared to controls, ENS mice show (i) poorer short-term memory, impaired learning and attention in the novel object recognition test and barnes maze; (ii) increased risk-taking and impulsivity in the elevated plus-maze, open field and light-dark box tests; and (iii) locomotor hyperactivity that develops over time but a blunted locomotor sensitization to cocaine. We are currently extending these results by examining the effects of ENS in the 5-choice serial reaction time test and the delay-discounting test, both of which are well-established for testing impulsivity and attention. ADHD is also highly comorbid with substance abuse. We found that ENS mice develop a stronger conditioned place preference for cocaine compared to controls, suggesting that they may have a higher addiction liability. Interestingly, our behavioral observations are specific to the developmental period as mice exposed to ENS as adults were not affected. At the cellular level, we found that ENS leads to a decrease in neurogenesis in the dentate gyrus of the hippocampus and an increase in miniature excitatory postsynaptic currents in the basal amygdala and nucleus accumbens shell. We are currently pursuing experiments to further characterize these cellular effects. In today's increasingly complex technological age, children face a tremendous amount of sensory stimulation that could interact with genetic predispositions to produce detrimental effects on behavior and brain function. Indeed, observational studies have shown an association between excessive stimulation in young children and subsequent memory and attentional problems. Thus, ENS may serve as a useful model to study the influence of environmental factors on vulnerability to ADHD and its underlying etiology.

Disclosures: S. Ravinder: None. **J.S.B. Ramirez:** None. **D. Christakis:** None. **J.M. Ramirez:** None. **S. Ferguson:** None.

Poster

685. Developmental Disorders: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 685.18/I3

Topic: C.06. Developmental Disorders

Support: FAPESP 2014/16711-6

FAPESP 2014/15018-5

Title: Dynamics of Inositol 1,4,5-triphosphate receptor type 1 (IP3R1) in neonatal anoxia

Authors: *J. M. IKEBARA, M. V. DAMICO, D. S. CARDOSO, F. A. DOS SANTOS, G. S. V. HIGA, S. H. TAKADA, A. H. KIHARA;
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Abstract: The neonatal anoxia is one of the most common causes of morbidity and mortality in neonates. The longlasting sequelae include motor deficits, behavioral and sensory and /or cognitive impairments. The oxygen deprivation causes loss of ionic homeostasis resulting in calcium overloading and changes of calcium waves patterns, which are responsible for initiating the cell death in neuronal tissue. Inositol 1,4,5-triphosphate receptors (IP3Rs) are important calcium channels which regulate cellular calcium homeostasis and dynamics at the level of the endoplasmic reticulum (ER). Furthermore, the IP3Rs can be present in clusters or individually and this property alters of the way that calcium is released. Into a IP3R1 cluster, the released calcium forms puffs, and when IP3R is isolated, the released calcium forms blips. The IP3R1 is widely expressed in brain and spinal tissue and plays a pivotal role in these processes by mediating calcium flux from the ER into the cytosol and mitochondria. The aim of this study was to identify possible changes in the expression and dynamic of IP3R1 in endoplasmic reticulum caused by anoxic insult in neonatal rat hippocampus. In this work were used Wistar rats (Rattus norvegicus) for anoxia and sham groups. Anoxia was performed in P1/P2 neonates according to described system of neonatal anoxia, composed by 25 minutes of 100% nitrogen gas exposure at 37°C (Takada et al., 2011). We measured genic expression 24 hours after neonatal anoxia (by real-time PCR) and analysis showed a decreased genic expression of IP3R1 in anoxia treated animals. It suggests that probably there is a compensatory mechanism after an intracellular calcium overload in order to restore the calcium homeostasis and prevent the cell death. Interestingly, it was observed differences in subcellular location of immunolabeled hippocampal cells in CA1 area in anoxia animals. Nucleus was labelled in anoxia group while in sham group the labelling was distributed throughout the cytoplasm. These results showed that neonatal anoxia altered the distribution and genic expression of IP3R1 and probably may interfere in calcium release patterns, providing bases for future therapeutic approaches aiming the normalization of IP3R1-mediated calcium release.

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Poster

685. Developmental Disorders: Animal Models II

Location: Hall A

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Topic: C.06. Developmental Disorders

Title: Identification of brain morphology defects in *Drosophila* CASK mutants

Authors: *J. TELLO, W. GRONENBERG, L. RESTIFO;
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Abstract: CASK encodes a highly conserved multi-domain scaffolding protein with major roles in brain development and function. CASK contains a guanylate kinase and selective protein-binding domains (PDZ and SH3) that define the MAGUK family; the amino-terminal CaM kinase-like domain has no enzymatic activity. CASK is primarily found in neurons, localized to both pre- and post-synaptic zones, as well as to the nucleus. Mutations in human CASK cause three distinct X-linked intellectual disability disorders, the most severe of which causes microcephaly with pontine-cerebellar hypoplasia and ataxia. To study the role of CASK in brain development, we used the recessive *Drosophila* CASK mutation, Δ18, a small deletion that eliminates all full-length CASK protein, compared with its specific genetic control, Ex33. Like the human patients, CASK-mutant *Drosophila* have impaired motor coordination that resembles ataxia. We analyzed brain morphology of Δ18 homozygous flies by histology of serial plastic-embedded sections. Estimated brain volume was reduced by 25% in Δ18 homozygotes, with the volume of different brain structures appearing to be reduced proportionately. Higher-resolution analysis of individual brain regions, such as the mushroom bodies and central complex, is underway. To investigate the cellular basis of the "small-brain" phenotype, we used primary cultures prepared from the CNS of *Drosophila* larvae to examine neuronal morphogenesis *in vitro*. Although the CNS of CASK mutants was visibly smaller than that of control larvae, neuronal cell recovery after dissociation was comparable. Neurite-arbor size and shape were quantified using NeuronMetricsTM software for semi-automated image analysis. CASK-mutant CNS neurons extended smaller arbors, with significant reductions in length, higher-order branch number, and territory, whereas branch density was increased. This "mini-arbor" defect suggests that the CASK-mutation-associated phenotypes, "small-brain" in flies and microcephaly in children, are due to impaired extension of dendritic arbors and axonal projections, rather than to

a reduction of neuronal numbers. These findings support the use of the *Drosophila* genetics system to model human brain disorders, in particular to study the functional consequences of specific human CASK mutations.

Disclosures: J. Tello: None. **W. Gronenberg:** None. **L. Restifo:** None.

Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NIH Grant NS091866

Title: Impaired plasticity in adult visual cortex following neonatal hypoxia

Authors: E. W. ATKINSON, H. ZHANG, *J. E. COLEMAN;
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Abstract: Premature infants are susceptible to diffuse white matter injury (DWMI), reductions in cortical and thalamic gray matter volume, and axonal injury. Consequently, children born premature are at an increased risk for long-term cognitive and behavioral deficits. Chronic hypoxia is a major risk factor for preterm brain injury and causes DWMI, ventriculomegaly, and behavioral deficits in rodents exposed to chronic sublethal hypoxia (CSH) as neonates. Although neuroimaging studies in children born premature suggest a link between anatomical abnormalities and the cognitive, learning and sensory processing deficits observed later in life, relatively little is known about the underlying pathophysiological mechanisms. We hypothesize that neonatal hypoxic brain injury disrupts the formation of microcircuitry in the cerebral cortex, which in turn impairs experience-dependent plasticity throughout life. To test our hypothesis, we characterized a robust form of adult experience-dependent plasticity in mouse primary visual cortex (V1), called stimulus-selective response potentiation (SRP), after exposure to CSH (10% O2, hypoxic, Hx) or room air (20% O2, normoxic, Nx) from postnatal day (P) 3 to P12. We used visually evoked potentials (VEPs) to assess basic visual function and SRP in adult (P40-60) Nx and Hx mice. We found that the intrinsic contralateral bias, visual acuity, and contrast sensitivity were normal in Hx mice. Following SRP induction, both Nx and Hx showed a significant potentiation of VEP amplitude to familiar (X deg) stimuli relative to novel (X + 90 deg) stimuli after 5 days, but the rate and overall magnitude was significantly reduced in Hx mice. Examination of the distribution of familiar and novel VEP amplitudes measured on day 5 in Hx

mice showed a scaled reduction in familiar, but not novel, VEP amplitudes. Furthermore, while VEPs elicited by familiar stimuli showed reduced variability in peak latencies when compared to VEPs elicited by novel stimuli, there was no difference between peak latencies measured in VEPs elicited by familiar and novel stimuli in Hx mice. These data provide support for our hypothesis by showing that cortical plasticity is impaired in adult Hx mice. Together, our findings warrant further investigation into the integrity of V1 microcircuitry in Hx mice and the behavioral consequences of reduced plasticity.

Disclosures: E.W. Atkinson: None. **H. Zhang:** None. **J.E. Coleman:** None.

Poster

685. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NIH grant K08

Title: Structural basis underlying therapeutic sensitive period for autistic behaviors in cerebellar mouse model of TSC

Authors: *P. TSAI[1], J. ELLEGOOD[2], J. LERCH[2], W. REGEHR[3], M. SAHIN[4];
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Abstract: Autism spectrum disorders are highly prevalent neurodevelopmental disorders that present a significant health care challenge. Despite the heightened prevalence, the underlying pathogenesis of autism spectrum disorders remains poorly understood. Clinically, cerebellar dysfunction has been implicated in autistic behaviors. Using a mouse model of tuberous sclerosis complex, we have demonstrated that cerebellar dysfunction is sufficient to generate autistic-like behaviors in mice and that early treatment with the mtor specific inhibitor rapamycin can prevent these abnormal behaviors. Here, we demonstrate that rapamycin treatment at a time corresponding to young adulthood can rescue abnormal autistic-like behaviors. Moreover, using voxel based morphometry and diffusion tensor imaging to investigate circuit based mechanisms underlying dysfunction and rescue, we also demonstrate underlying anatomic changes in mutant mice that - similar to behavioral disruption - can be rescued with adult onset rapamycin therapy.

Disclosures: **P. Tsai:** None. **J. Ellegood:** None. **J. Lerch:** None. **W. Regehr:** None. **M. Sahin:** None.

Poster

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Support: Brain Canada Grant

Title: Chronic intranasal oxytocin administration in the 16p11.2 mouse model has a marginal effect on behaviour and no effect on neuroanatomy

Authors: ***Z. BUCHWALD**[1], J. ELLEGOOD[1], E. ANAGNOSTOU[2], J. LERCH[1];
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Abstract: Introduction: Autism is a neurodevelopmental disorder characterized by social/communication deficits and repetitive behaviors. Oxytocin is a neuromodulator known for its ability to promote social behaviours and therefore, may be a promising therapeutic for autism. However, as autism is heterogenous, the treatment will likely only help with a subset of patients. To determine what might contribute to response susceptibility, we treated the 16p11.2 mouse model, representing one of the most common copy number variants found in autism, with oxytocin. Methods: To assess the efficacy of treatment, intranasal oxytocin (~6ul) was administered once a day, for 28 days, starting at 5 weeks of age to 17 male 16p11.2 mutant mice and 19 male littermate controls. On the third week of treatment, the mice were assessed for repetitive behaviours (as assessed by grooming), sociability (three chamber sociability task), anxiety and hyperactivity (open field), memory (novel object recognition), and learning and motor coordination (rotarod). Prior to the oxytocin treatment, and before and after behavioural testing, the mice underwent *in vivo* neuroimaging using MRI to gather information about any neuroanatomical changes. An additional *ex vivo* MRI was acquired at the end of the study. Results: Oxytocin treatment normalized ($p< 8.8e-5$) the repetitive behaviour deficits of the 16p11.2 mutant mouse and increased ($p<0.034$) their object recognition (memory), but had no effect on sociability, anxiety, or hyperactivity. Treated mutant mice fell off the rod quicker ($p<0.005$) than untreated mutants, indicating that oxytocin might exacerbate motor deficits. Treatment also had no significant effect on neuroanatomy. Discussion: Oxytocin is expected to increase social behaviours and memory, and decrease repetitive behaviours. Treatment had no

significant effect on neuroanatomy and, for the most part, did not enhance the behaviours of the 16p11.2 mouse, though it normalized a deficit in grooming and increased memory. Results indicate that oxytocin may not be a good treatment option for 16p11.2 mutant mice, and therefore, humans with the 16p11.2 mutation also may not respond positively to oxytocin treatment. Future directions involve looking at the response of multiple strains of autism-related mouse models, including 16p11.2, to several promising therapeutics used in human patients with autism, yielding the ability to establish a translational paradigm for predicting responders from non-responders.

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Poster

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Title: Impairment in spatial memory and alteration in excitatory synaptic transmission in Myosin9a heterozygous mice

Authors: ***E. VEZZOLI**[1,2], A. FOLCI[3], L. MURRU[3], S. BASSANI[3], L. PONZONI[1], F. LONGO[3], E. MORETTO[3], L. GEROSA[3], J. ZAPATA[3], D. BRAIDA[1], M. BÄHLER[4], M. SALA[1], M. FRANCOLINI[1,3], M. PASSAFARO[3];
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Abstract: Unconventional myosins are a superfamily of actin-based motors; among these, Myosins9, belong to a class formed by two members: Myosin9a (Myo9a) and Myosin9b (Myo9b). Myo9a was first identified and cloned in 1998 and it was demonstrated that it was enriched in rat brain (cerebellum, cortex and hippocampus) where its high level of expression suggested that it had a prominent role in the central nervous system functions (Chieregatti et al., 1998), a role that is however still elusive. In humans, mutations in Myosin9a gene were shown to be correlated to the Bardet-Biedl Syndrome, an autosomal recessive disorder characterized by a number of pathological features including intellectual disability. In mice, loss of Myo9a induced severe hydrocephalus; brains from early postnatal mice were characterized by stenosis of the ventral caudal third ventricle and the aqueduct. It was recently shown that hydrocephalus was

caused by a persistent activation of RhoA proteins as treatment with a specific inhibitor was able to reduce its formation new-born mice (Chieregatti et al., 2009). Here, we investigated the role of Myo9a in the hippocampal excitatory synapses, in Myo9a heterozygous male mice (C57BL/6) at four/six weeks of age. Ultrastructural analyses of the CA1 region of hippocampus showed that synapses of Myo9a+/- mice were characterized by an increase in the thickness of postsynaptic density (PSD). On the other hand, no differences were observed in the architecture of presynaptic terminals. In agreement with these results biochemical assay on Myo9a-/+ hippocampus homogenates showed increased levels of postsynaptic (PSD-95 and GluA2) but not presynaptic markers. We also found that Myo9a was enriched in the PSD, where it colocalized with postsynaptic markers and directly bind the C-terminus of the GluA2 AMPA receptor subunits. This last result suggested a possible involvement of Myo9 in AMPA receptor trafficking; a role in glutamate receptors dynamics that was further confirmed by electrophysiological recordings indicating that hippocampal neurons from Myo9a+/- mice showed an altered basal excitatory pattern characterized by increased amplitude and frequency of mEPSCs. Moreover in the CA3-CA1 synapses from acute slices from Myo9a+/- mice, the induction of Long Term Potentiation (LTP) was severely impaired and this impairment of LTP in *ex vivo* recordings was paralleled by a strong deficit in spatial learning and memory. From these data, we could conclude that the partial deletion of Myo9a, impaired spatial memory and altered excitatory transmission interacting with the AMPA receptors trafficking in the hippocampus.

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Poster

686. Developmental Disorders: Animal Models III

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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 cognitive impairment in a murine model of intraventricular hemorrhage in the preterm infant

Authors: A. SEGADO-ARENAS[1], C. INFANTE-GARCIA[2], J. RAMOS-RODRIGUEZ[2], S. LUBIAN-LOPEZ[1], *M. GARCIA ALLOZA[2];
[1]Hosp. Universitario Puerta del Mar, Cadiz, Spain; [2]Div. of Physiology, Sch. of Med., Cadiz, Spain

Abstract: Preterm birth (PTB) is defined as birth prior to 37 weeks of gestational age and affects over 10% of births worldwide. Also, the increase in the survival of the tiniest premature infants, has also raised the incidence of preterm-related pathologies. One of the most feared complications in PTB is the germinal matrix-intraventricular hemorrhage (IVH), affecting up to 30% of babies with extremely low birth weight (<1000 g) and 15% of those under 1500 g. We have developed a murine model of IVH by intracerebroventricular injection of 0.1U or 0.3 U of proteolytic enzyme collagenase to p7 CD1 mice. As soon as 24 h after the injections we observed dose-dependent intraventricular bleeding. We hypothesized that induced IVH had deleterious effects on cortical and hippocampal development. In order to further explore this possibility, mice were analyzed postmortem at p14 and at p70. Whereas brain weight was similar in all groups under study at p14, an slight reduction was observed in p70 mice treated with highest dose of collagenase. Brain morphology and atrophy were further assessed using cresyl violet staining. Bleeding was measured by Prussian blue staining and inflammatory processes were quantified by microglia immunostaining with anti-iba 1 antibody, both in the close proximity of ventricular hemorrhages, as well as in hemorrhage free areas. By p70, when behavioural assessment can be performed, we observed a dose-dependent cognition impairment in collagenase treated mice. Spatial memory was significantly impaired in the Morris water maze test, both in 0.1 U and 0.3 U treated mice. Episodic memory was also significantly impaired in collagenase injected mice in a dose-dependent manner. Altogether, our data suggest that our model of induced IVH in PTB might be useful in the study of the related pathology and prognosis. Our results may guide us to better understand the physiopathology involving IVH and help us in the search for new therapeutic targets. 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

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Support: Autism Science Foundation

NIH Grant HD071998

Title: Developmental exposure to fluoxetine alters social behavior and arginine vasopressin receptor binding in prairie voles (Microtus ochragaster) associated with Autism Spectrum Disorder

Authors: *M. C. PALUMBO[1,2], R. H. LARKE[1,2], S. M. FREEMAN[2], K. L. BALES[2,1]; [1]Univ. of California, Davis, Davis, CA; [2]California Natl. Primate Res. Ctr., Davis, CA

Abstract: The neuropeptide hormone arginine vasopressin (AVP) is involved in social behavior, pair bonding, and stress responses in the socially monogamous prairie vole (Microtus ochrogaster). Exposure to a developmental selective serotonin reuptake inhibitor (SSRI) *in utero* may be linked to the prevalence of Autism Spectrum Disorder (ASD). As a translational model of developmental pre-and postnatal SSRI exposure, twenty female prairie voles were treated with 5mg/kg subcutaneous fluoxetine or saline solution daily throughout pregnancy to weaning. The SSRI-exposed pups were behaviorally assessed to examine anxiety-like and attachment behaviors using the partner preference (PPT), elevated plus maze, open field, and alloparental care tests. Brains were collected 24 hours after behavioral testing. Quantitative receptor autoradiography was used to measure vasopressin 1a receptor (V1aR) binding density in the medial amygdala, lateral septum, and ventral pallidum. Analyses (N=69) indicate developmental fluoxetine exposure decreases V1aR density in the medial amygdala ($F_{1,47}=4.2$, $p<.05$) and voles with lowered V1aR density in the medial amygdala spent a longer duration in the empty testing chamber ($F_{1,2}=39.82$, $p=.02$) in the PPT. Females with decreased V1aR density in the medial amygdala ($F_{1,2}=70.51$, $p=.01$) spent the most amount of time in the empty testing chamber during the PPT. These results suggest changes to V1aR density in the medial amygdala alter sociality and may mediate the increased risk of ASD. This research may help to explain the relationship between SSRI exposure during pregnancy and neural and behavioral alterations associated with ASD.

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Poster

686. Developmental Disorders: Animal Models III

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Title: A bidirectional threshold of ERK/MAPK signaling regulates axonal outgrowth in developing corticospinal neurons

Authors: *L. XING[1], G. R. BJORKLUND[2], X. LI[1], Y. WU[1], W. D. SNIDER[1], J. M. NEWBERN[2];
[1]Neurosci. Ctr., UNC Chapel Hill, Chapel Hill, NC; [2]Sch. of Life Sci., Arizona State Univ., Tempe, AZ

Abstract: RASopathies represent a large family of human neurodevelopmental disorders caused by aberrant ERK/MAPK signaling due to germline mutations in regulators and components of the canonical Ras/Raf/MEK/ERK signaling pathway. However, the cellular and molecular mechanisms underlying the pathogenesis of RASopathies are largely unclear. Here, using a mouse genetic approach, we have delineated the specific effects of both loss and gain of function of ERK/MAPK signaling in the development of long-range cortical projection neurons. We found that inactivation of ERK/MAPK signaling in developing cortical excitatory neurons resulted in a marked loss of Ctip2 expressing, subcerebrally-projecting neurons in sensorimotor layer V and a dramatic reduction in the size of the corticospinal tract (CST) by P14. ERK/MAPK signaling is not required for initial neuronal specification as, at P0, Ctip2-expressing layer V neurons were present in normal numbers. However, the entry and extension of CST axons into the cervical spinal cord was markedly reduced at P2. Subsequently, many layer V neurons underwent apoptosis as evidenced by the presence of activated Caspase-3 labeled cells and the association of microglia with degenerating neurons. We hypothesize that CST axon growth failure may be due to reduced ability to respond to IGF1 which we have found is intensely expressed in the ventral medulla from E18-P14. Surprisingly, hyperactivation of ERK/MAPK also resulted in diminished CST size in the spinal cord dorsal funiculus in adult mice, but the number of Ctip2 expressing layer V neurons was not altered. A striking decrease in the initial extension of corticospinal afferents into the cervical spinal cord was evident at P3-4. Viral tracing of layer V neuronal projections provided further support for reduced spinal cord

innervation in adults. Interestingly, reduced extension the spinal cord was associated with enhanced CST axon targeting and branching in the medulla. In contrast, neither loss nor gain of function of ERK/MAPK signaling had an overt effect on the survival and axonal growth of callosal projection neurons in layer II/III in early postnatal developmental stage. Collectively, our data reveal that a bidirectional threshold of ERK/MAPK signaling is critical to the regulation of elongation and branching of corticospinal axons. Abnormalities in axonal connectivity of layer V neurons might contribute to the pathogenesis of neurodevelopmental syndromes associated with pathological ERK/MAPK activity.

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Poster

686. Developmental Disorders: Animal Models III

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JSPS KAKENHI Grant Number 26860851 to SM

Title: Electron microscopic evaluation of myelination in the sensorymotor cortex in a neonatal hypoxic-ischemia model that has hindlimb motor dysfunction without neuronal loss

Authors: *Y. UEDA[1,1], H. TAKASE[2], S. MISUMI[3], A. ISHIDA[3], C.-G. JUNG[3], H. HIDA[3]; [1]Neurophysiol & Brain Sci., [2]Core Lab., [3]Neurophysiol and Brain Sci., Nagoya City Univ. Grad Sch. Med. Sci., Nagoya, Japan

Abstract: Developing white matter injury (DWMI) caused by perinatal hypoxia-ischemia (H-I) in preterm infants is associated with permanent neurodevelopmental disabilities such as paralysis and cognitive dysfunction. We established a DWMI model rat that received H-I (right common carotid artery occlusion and 6% hypoxia for 1 hour) at P3. Our DWMI model showed less hindlimb motor coordination and altered motor mapping in the sensorymotor cortex. Less neuronal damage was observed in the cortex and white matter: few apoptotic neuronal cell death, few degenerating cells in Argyrophil-III silver staining, comparable number of cortical projecting neurons and pyramidal neurons, and comparable number of GABAergic cells. We also revealed that the intensity of MBP staining was lower in the layer II-III of the sensorymotor

cortex. To clarify the reason for hindlimb dysfunction and the mechanism of mapping change in our DWMI model, we investigated myelination in the motor cortex (M1) and the sensory cortex (S1) using electron microscopy (EM). DWMI rats were perfused with the fixative, and ultrathin sections of each hindlimb area were observed under EM (x 5,000). The number of myelinated axons and their g-ratio was evaluated in the layer II-III, the layer V and corpus callosum (CC). In frontal section assessment, No significant difference in the number of myelinated axons in M1 and S1 was detected in the layer II-III where the intensity of MBP was less. In addition, no significant difference in g-ratio of myelinated axons was there in M1 and S1. However, evaluation from the horizontal section of the layer II in M1 showed increased myelinated axons on the DWMI side. Data suggest that myelination in the layer II is altered in our DWMI model, which could explain the reason for the hindlimb dysfunction and the mechanism of mapping change in the cortex.

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Poster

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Topic: C.06. Developmental Disorders

Support: KAKENHI (256400002)

Title: Crosstalk between paternal Pax6 mutation and aging accelerates vocal communication

Authors: ***R. KIMURA**, K. YOSHIZAKI, K. KOIKE, H. INADA, N. OSUMI;
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Abstract: In rodent, advanced paternal age induces some abnormal behaviors, and how paternal aging affects phenotypes of their offspring is an intriguing issue. We previously demonstrated that the offspring derived from old sires showed significant decrease in average number of ultrasonic vocalization (USV) that is known as communicative call of mice. Histological analysis suggested that this trans-generational effect of advanced paternal age had its molecular basis on epigenetic changes during spermatogenesis. Here, we report that paternal Pax6 mutation accelerates the decrease in USV calls caused by advanced paternal age. To examine the influence of paternal Pax6 mutation and paternal aging to their offspring, spontaneous Pax6 mutant (Sey) sire mice at each stage of young (3-month-old), middle-aged (6-8-month-old), and old (>12-

month-old) were mated with young (3-month-old) wiled-type (WT) females. Their offspring were separated from the dams at postnatal-day 6 and the number of USV calls was measured during 5 minutes separation. Although the number of USV calls was comparable between Sey offspring and WT litter when their Sey sire was young, Sey offspring derived from middle-age Sey sire showed significant decrease in the number of USV calls compared to WT litter. The number of USV calls was decreased in both genotypes of offspring when their Sey sire was old. We also found the expression of Pax6 in spermatocyte, suggesting that Pax6 haploinsufficiency accelerates epigenetic changes in spermatocyte caused by advanced paternal age. Semiquantitative analysis demonstrated that histone H3 K9 di-methylation and K79 di-and tri-metylation level in spermatocyte were up regulated in Sey spermatocytes. This increase of H3K79 tri-methylation was also observed in mature sperm. Since there was a significant association between the level of H3K79 tri-methylation in sperm and the number of USV in their offspring in WT, it is possible that paternal Pax6 mutation accelerates vocal communication deficit in offspring caused by paternal aging through the transgenerational mechanism including H3K79 tri-methylation in spermatocytes.

Disclosures: R. Kimura: None. **K. Yoshizaki:** None. **K. Koike:** None. **H. Inada:** None. **N. Osumi:** None.

Poster

686. Developmental Disorders: Animal Models III

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 686.08/I14

Topic: C.06. Developmental Disorders

Support: NSERC of Canada

AIHS

Title: The effects of prenatal exposure to valproic acid on the development of juvenile-typical social play in rats

Authors: *S. RAZA, B. T. HIMMLER, S. M. HIMMLER, A. HARKER, B. KOLB, S. M. PELLIS, R. GIBB;
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Abstract: Autism is a severe neurodevelopmental disorder characterized by qualitative impairments in social behavior, communication, and aberrant repetitive behaviors. A major focus

of animal models of autism has been to mimic the social deficits of the disorder. The present study assessed whether rats exposed prenatally to valproic acid (VPA) exhibit deficits in social play as juveniles that are consistent with the social deficits seen in autism. Dams were exposed to an acute dose of VPA on gestational day 12.5. Later, the playful interactions and associated ultrasonic vocalizations (USVs) of the juveniles were examined. It was predicted that VPA-treated rats should play less than the controls. Characteristic of neurobehavioral insult at this early age, the VPA-treated juveniles exhibited significant increases in the frequency of body shakes and sexual mounting, but played at the same frequency as the controls. However, when playing, they were less likely to use tactics that facilitated bodily contact and vocalized less. These data suggest that prenatal VPA exposure disrupts some aspects of being able to communicate effectively and engage partners in dynamic interactions - deficits that are consistent with those seen in autism.

Disclosures: **S. Raza:** None. **B.T. Himmler:** None. **S.M. Himmler:** None. **A. Harker:** None. **B. Kolb:** None. **S.M. Pellis:** None. **R. Gibb:** None.

Poster

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Support: Hungarian Governmental Grant (“Ernyő” 2013-2016)

Title: Complex behavioral and functional phenotype in a rat prenatal valproate model of autism

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Abstract: Autism spectrum disorder is a highly prevalent developmental disorder that is characterized by deficits in socio-communication, increased stereotyped behaviors and compromised cognitive processes. Human fetal exposure to valproate (VPA), a widely-used anti-epileptic drug has been shown to increase the risk of developmental disorders including autism spectrum disorder. Literature data indicate that VPA administration to pregnant rodents may also lead to autistic-like behavioral elements in the offspring and is used as an animal model of autism. In the present study, Sprague-Dawley rat dams were treated with 400 mg/kg sodium

VPA (sc.) or the same volume of saline vehicle (given the same route) on gestational day 12.5 and behavior of the offspring was investigated in a battery of tests. In accordance with an autistic-like phenotype, ultrasonic vocalization after maternal deprivation was reduced in the VPA-treated pups and adult offspring showed a major defect in social memory measured in the social discrimination set-up of the 3-chamber apparatus. Social preference in the 3-chamber assay, juvenile social play, spontaneous locomotor activity and full circadian activity in adulthood were mildly affected or unaltered in the *in utero* VPA-exposed rats. Animals were also investigated in a rodent-dedicated magnetic resonance imageing (MRI) apparatus to reveal potential underlying anatomical, resting state and/or functional alterations. MRI done in an anatomical mode indicated a reduction in both white and grey matter in the VPA group. Resting state MRI did not show a difference between *in utero* VPA and saline treated offspring. In a functional MRI set-up air-puff stimulation of the whisker pad induced a hyperactivation of the somatosensory cortex in the prenatally VPA-treated offspring compared to controls, supporting the excitatory/inhibitory imbalance theory of autism spectrum disorder. In conclusion, our results suggest that VPA treatment in Sprague-Dawley rats results in a complex phenotype with a number of behavioral and functional alterations that are compatible with the notion of prenatal VPA administration as a pharmacological model of ASD. This work was partially supported by a Hungarian Governmental Grant ("Ernyő" 2013-2016).

Disclosures: **G. Levay:** A. Employment/Salary (full or part-time):; Gedeon Richter Plc. **C. Csolle:** A. Employment/Salary (full or part-time):; Gedeon Richter Plc. **R. Kedves:** A. Employment/Salary (full or part-time):; Gedeon Richter Plc. **K. Saghy:** A. Employment/Salary (full or part-time):; Gedeon Richter Plc. **K. Kordas:** A. Employment/Salary (full or part-time):; Gedeon Richter Plc. **A. Varga:** A. Employment/Salary (full or part-time):; Gedeon Richter Plc. **T. Spisak:** A. Employment/Salary (full or part-time):; Gedeon Richter Plc. **D. Gajari:** A. Employment/Salary (full or part-time):; Gedeon Richter Plc. **V. Roman:** A. Employment/Salary (full or part-time):; Gedeon Richter Plc.

Poster

686. Developmental Disorders: Animal Models III

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Support: Canadian Institutes of Health Research (CIHR)

Fonds de recherche du Québec – Santé (FRQ-S)

Foundation of Stars

Heart and Stroke Foundation

Title: Live group B Streptococcus-induced maternal immune activation: gender dichotomic chorioamnionitis and autistic-like traits in male offspring

Authors: *M.-J. ALLARD[1], J. BERGERON[1], M. DESCOTEAUX[1], L. TREMBLAY[1], M. LEPAGE[1], L.-C. FORTIER[1], C. POYART[2], G. SÉBIRE[1,3];
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Abstract: Background Group B *Streptococcus* (GBS) is a commensal bacterium colonizing 15 to 30% of healthy pregnant women. GBS is one of the major causes of chorioamnionitis, associated to preterm delivery and cerebral injuries in the newborn. Our hypothesis is that GBS-induced maternal immune activation has a deleterious neurodevelopmental impact on the offspring. Using a new preclinical rat model, our goal is to study the impacts of GBS-induced gestational inflammation on the developing brain and its behavioral outcomes. **Methods** Dams were inoculated at gestational day (G) 19 with live serotype Ia GBS (10^8 CFU). Caesarian sections were performed at G21 and G22 to collect maternal and fetal blood, placentas and fetal brains. The maternofetal inflammatory response was studied by ELISA and immunohistochemistry. Brains were collected at postnatal (P) 40 for histological studies. Magnetic resonance imaging (MRI) and diffusion tensor imaging were executed on 69 to 83 days old anesthetized rats. Behavioral tests were performed from P7 to P40 to assess communication, sociability, sensory integration and exploratory abilities. **Results** GBS-exposed dams displayed gender dichotomic chorioamnionitis characterized by a higher infiltration of polymorphonuclear cells in male, compared to female fetuses. Increased titers of interleukine-1β were detected in the maternal blood and in male fetuses' blood following GBS inoculation. At P40, GBS-exposed males showed a reduced thickness of frontal cortex and of periventricular white matter, and an enlargement of lateral ventricles, which was also visible on MRI. Trend toward a decreased mean fractional anisotropy, reflecting dysconnectivity of white matter fibers, was observed in the genu and body of corpus callosum in GBS-exposed *vs* control male rats. GBS-exposed males, but not females, showed autistic-like behavior: defective communication, social behavior impairments, impaired sensory integration, and hyperactivity, as compared to male controls. **Conclusions** Exposure to live GBS induces maternal immune activation resulting in neurodevelopmental and behavioral abnormalities recapitulating those of human autism spectrum disorders, including gender dichotomy and neurobehavioral phenotype. White matter dysconnectivity will be further characterized by ongoing studies. Further neurobehavioral characterization and inflammatory mechanistic studies of this new preclinical animal model will help to target future neuroprotective strategies.

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Poster

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Support: NIH training grant T32 007051

DOD W81XWH-13-0306

Title: Effects of perinatal administration of antidepressants on behavior of the adult offspring in Sprague-Dawley rats

Authors: *J. NELMS SPROWLES[1,2], J. R. HUFGARD[1,3], A. GUTIERREZ[1,3], R. A. BAILEY[1,3], S. A. JABLONSKI[1,2], M. T. WILLIAMS[1,2,3], C. V. VORHEES[1,2,3];
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Abstract: During fetal life, the brain rapidly develops and undergoes numerous processes such as cell fate determination, neurogenesis, migration, synaptogenesis, apoptosis, and pruning. Throughout this critical period, the brain is susceptible to insult from exposure to exogenous factors, including drugs, which may negatively impact brain development. Antidepressants (ADs) modify neurotransmitter reuptake and interact with receptors in the brains of adults; however, the effects of such activity on early brain development are not well understood. A recent study suggested an increase in autism spectrum disorder (ASD) in children whose mothers took selective serotonin reuptake inhibitors (SSRIs) while pregnant (Croen et al., 2011). Another study reported deficits in learning and memory after exposure to an SSRI from P11-20 in rats (Schaefer et al., 2013). The present study investigated whether perinatal exposure to antidepressants affects long-term behavioral outcomes. Sprague-Dawley dams were randomly assigned to one of six treatment groups: Citalopram (10 mg/kg or 5 mg/kg; SSRI), Fluoxetine (5 mg/kg; SSRI), Buproprion (15 mg/kg; NDRI), Valproic Acid (500 mg/kg - given once on E12 as a positive control for ASD-like behavior), or Saline. Dams were treated by subcutaneous injection twice daily (6 h apart) from E6-21, and pups were dosed directly from P1-20. Behavioral testing began on approximately P60. One male/female pair from each litter was tested in the Cincinnati water maze for egocentric learning. Another pair received the Morris water

maze to assess allocentric learning and memory. A third pair received a series of tests: elevated zero maze, open-field, marble burying, acoustic startle response with prepulse inhibition, sociability/social preference, radial water maze, and forced swim. Rats underwent testing in a straight water channel prior to maze testing to assess motor function, motivation to escape, and to teach them how to escape on a hidden platform. A fourth pair was sacrificed for immunohistochemistry and HPLC analyses. Preliminary data analyses show significant treatment effects in open-field (Cit-10 group was hypoactive compared with Sal, $p<0.05$) and marble burying (Cit-10 rats buried more marbles than Sal rats, $p<0.05$, suggesting increased compulsive behavior). Early results suggest that AD exposure during early development results in altered adult behavior in the offspring with possible implications for the safety of these drugs when used during pregnancy.

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Poster

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Support: NIH grant HG000330

Title: Human-Mouse:Disease Connection, utilizing mouse models to gain insights on autism

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Abstract: The Human-Mouse: Disease Connection (HMDC, www.diseasemodel.org) is a translational tool developed by the Mouse Genome Informatics resource (MGI, www.informatics.jax.org) to aid researchers in identifying candidate disease genes and finding mouse models of diseases and disorders such as autism. Autism spectrum disorder encompasses a group of neurodevelopmental disorders with a strong genetic basis, characterized by social interaction and communication difficulties, repetitive behaviors, and other health issues. The use of mouse models is key for gaining insights on the etiology, molecular and cellular mechanisms, behavioral phenotypes, and environmental factors that contribute to autism. In addition, well-defined mouse models showing core autism phenotypes can help discover new therapies and be

used to test potential treatments. The HMDC integrates mouse and human genomic, phenotypic, and disease information, including over 1,300 OMIM-derived human diseases with one or more mouse models, phenotypes from more than 54,800 mouse genotypes, and high-throughput phenotyping data of mouse knockout mutations from the International Mouse Phenotype Consortium (IMPC), in easy to navigate and interactive grids and tables. Investigators can identify experimental mouse models of autism based on OMIM disease associations by searching for disease terms. The results of a search on "autism" (as of May 2015) show 32 mutant mouse genes described to produce autism phenotypes. Of these, three orthologs are identified in humans as autism genes. These mouse mutants are robust models that can be used for testing therapeutic agents. An additional 23 of the mutant mouse genes have human orthologs and are potential autism candidate genes that researchers may want to explore further in patients. The results also show 7 human genes implicated in autism for which mouse orthologs are known, but no data exists to determine if mutants in these mice will display autism phenotypes. For example, mutations in *Slc9a9* or *Rpl10* are candidates for mouse autism models, but as these mutants exist only in ES cell lines, no phenotypic data are available. Phenotyping mice created from these mutant ES cell lines may yield new models. In HMDC, investigators can also prioritize candidate variants from whole genome sequencing of autistic patients and candidates within a genomic region based on reported phenotypes and disease associations. The HMDC portal eases the translation between human and mouse data. We will illustrate how users can exploit the wealth of mouse data to further research on complex neurodevelopmental diseases such as autism and to build better mouse models of disease.

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Poster

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Support: JSPS KAKENHI (26430020)

JSPS KAKENHI 26860851)

Title: Weaker myelin expression in layer II-III of the sensorymotor cortex in a neonatal hypoxic-ischemia model that has hindlimb motor dysfunction without neuronal loss

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Abstract: Developing white matter injury (DWMI) caused by perinatal hypoxia-ischemia (H-I) in preterm infants is associated with permanent neurodevelopmental disabilities such as paralysis and cognitive dysfunction. We reported a DWMI model rat that received H-I (right common carotid artery occlusion and 6% hypoxia for 1 hour) at P3. Our DWMI model showed less hindlimb motor coordination and altered motor mapping in the sensorymotor cortex. Less neuronal damage was observed in the cortex and white matter: few apoptotic cell death in NeuN positive cells, few degenerating cells in Argyrophil-III silver staining, comparable number of SATBII and Ctip2 positive cortical projecting neurons and Fluoro-gold positive pyramidal neurons, and comparable number of GABAergis cells after H-I. To clarify the reason for hindlimb dysfunction in our DWMI model, we challenged to know the myelination in the motor cortex (M1) and the sensory cortex (S1) using myelin basic protein (MBP) staining. Weaker MBP staining was apparently shown in both M1 and S1 in H-I side. Quantitative assessment in a selected area of layer II-III and layer IV-V that was defined by STABII staining was performed using Image J software: the intensity was digitalized at 16 bit, appropriate threshold was set, and the pixel above the threshold was quantified. It found that the intensity of MBP staining was lower in the layer II-III both M1 and S1 although it was same level in the layer V-VI in both areas. Data suggest that less MBP in the layer II-III in our DWMI model rat might explain hindlimb motor dysfunction without neuronal loss in the sensory-motor cortex.

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Poster

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Topic: C.06. Developmental Disorders

Title: Using the valproate-induced autistic spectrum disorder rat model to study the effect of minocycline on social emotional behavior

Authors: *P.-S. CHEN[1], M.-C. NG[2], C.-C. WANG[3], H.-C. LIN[4];
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Abstract: Recent studies have implicated immune dysregulation or inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures in ASD. Among then, strong evidences suggest neuroinflammatory and immunological dysregulation is important in the pathogenesis of ASD Prominent microglia activation in certain brain regions has been discovered in ASD patients. Previously, we have demonstrated that amygdala dysfunction may play a key role in the pathogenesis of ASD by using the valproic acid (VPA)-induced ASD rat model. In the current study, we treated the model with minocycline to study the effects on neuroinflammation, emotion brain activity and social phenotypes. The results showed, minocycline (50mg/kg) treatments for 14 days could increase social interaction and improved fear memory extinction in these VPA-exposed offspring. The results suggested the possible role of emotion brain neuroinflammation in the pathogenesis and the intervention of ASD. This work may shed light on our understanding of ASD and ultimately pave the way for developing safe and effective therapeutic strategies.

Disclosures: **P. Chen:** None. **M. Ng:** None. **C. Wang:** None. **H. Lin:** None.

Poster

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Topic: C.06. Developmental Disorders

Title: Mechanisms and rescue strategy for complex neuropsychiatric disorders in inborn error of peptide metabolism

Authors: *M.-H. KIM, Y.-S. BAE, S. YOON, W. SONG, S. LEE;
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Abstract: Inborn errors of metabolism (IEMs) are commonly accompanied by neurodevelopmental disorders. However, pathological mechanisms and pharmacological treatments for neurological symptoms in most IEMs are still unknown. Here we report that restoration of NMDAR (N-methyl-D-aspartate receptor) activity rescues neurodevelopmental disorders in mice lacking aminopeptidase p1 (Xpnpep1-/-). Mice deficient for aminopeptidase p1, a proline-specific endopeptidase encoded by Xpnpep1 gene, display hyperactivity, epileptic EEG rhythms, and impaired learning. These behavioral impairments were associated with

abnormal NMDAR signaling in the hippocampus. Restoration of NMDAR signaling by pharmacological intervention ameliorated behavioral and cognitive impairments caused by aminopeptidase p1-deficiency. These results indicate that metabolic dysfunction in Xpnpep1-/- neurons results in perturbation of NMDAR homeostasis and provide a potential therapeutic strategy to alleviate neurodevelopmental disorders in IEM caused by aminopeptidase P1-deficiency.

Disclosures: **M. Kim:** None. **Y. Bae:** None. **S. Yoon:** None. **W. Song:** None. **S. Lee:** None.

Poster

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Support: FACEPE APQ-1026.4-09/12

FACEPE IBPG-1241-2.08/12

Title: Gender difference in maternal low protein-diet offsrings: Oxidative stress evaluation

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Abstract: Epidemiological and experimental evidences indicates that insults during fetal and neonatal developmental period can increases the risk in the offspring to develop several chronic diseases including cardiovascular diseases and hypertension. In association, low-protein diet induces disruption in biochemical milieu in many tissues also increasing the risk of several diseases. Previous studies have shown that the estrogen has an important role on brain protection and its presence may be related to the decrease in the occurrence of diseases. Our hypothesis is that female offsprings are more protected than male that received low-protein diet during critical period of brain develop regards to the oxidative stress. Wistar offsprings (male, n=17 and female n= 15) were divided into two groups according mothers' diets: control (NP, 17 % of casein) and low-protein (LP, 8 % of casein) groups. There was no difference in lipid peroxidation in both a groups. We found a decrease in protein oxidation in females-LP group when compared to males-LP group (approximately 100%). The superoxide dismutase-SOD activity showed no difference

between groups. Related to catalase-CAT activity we observe an increase in in females-LP compared to males-LP (approximately 60%). The levels of reduced glutathione-GSH increased and decreased of levels of oxidized glutathione-GSSG in females-LP compared to males-LP group (20% and 45% approximately, respectively). Our results show that females are less susceptible to oxidative stress than males when submitted to protein malnutrition early in life.

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Poster

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Title: The role of Slitrk-1 gene in striatal cholinergic interneurons in behavioral manifestations mimicking Tourrette syndrome

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Abstract: The Slitrk-1 (Slit and NTrk-like family 1) gene is one of vulnerable risk genes in Tourette syndrome (TS), a chronic neuropsychiatric disorder with multiple motor and phonic tics regarded as stereotyped behaviors. In the striatum of mammals, Slitrk-1 is highly expressed in early childhood but gradually disappeared in adulthood except cholinergic interneurons, which are the only striatal neurons with persistent expression of Slitrk-1 into adult stage. A postmortem study had shown that adult TS patients had less cholinergic interneurons in the striatum than normal subjects, suggesting the contribution of Slitrk-1 in maintaining normal function of striatal cholinergic interneurons in adults. We, therefore, hypothesized that silencing Slitrk-1 in the striatum of adult mice can be an animal model of TS by impairing the function of striatal cholinergic interneurons specifically, leading to certain stereotyped behaviors. Slitrk-1 siRNA or non-targeting scramble siRNA (Accell siRNA) was bilaterally injected (0.5 μg in 0.5 μl) into the dorso-medial striatum (A:-0.74mm, L:±1.6 mm, D: 3.5 mm) of C57Bl/ 6J mice (8-10 weeks). A serial analysis of mice behaviors and prepulse inhibition (PPI), a TS endophenotype, was performed after injection of siRNA for 72 hours. Mice with Slitrk-1 siRNA injection (Slitrk-1-KD, n=4) showed significantly more stereotyped behaviors, compared to those with scramble

siRNA (n=4), and these stereotyped behaviors were suppressed significantly by haloperidol (0.3mg/kg, i.p.), an effective anti-tic agent by blocking D2 receptors (n=4). Both groups (n=6) displayed similar spontaneous locomotor activities and rearing numbers. The Slitrk-1-KD group had more apomorphine-induced climbing behaviors (n=6) and impaired PPI (n=3), as compared with the scramble group (n=3). Western blot analysis shows 69.4% decrease of the Slitrk-1 protein in the bilateral striatum of Slitrk-1-KD mice (n=4), as compared to wild type mice, but no change in the scramble group. Immunofluorescence study of brain slices in Slitrk-1 group (n=8) also revealed 69.6% decrease in the number of striatal neurons co-expressing Slitrk-1 and choline acetyltransferase, compared with scramble group. These results support Slitrk-1 deficit plays a role in the pathogenesis of TS and selective knockdown Slitrk-1 gene in the striatal cholinergic neurons of adult mice, inducing haloperidol-sensitive excessive stereotypy behaviors and impaired PPI, can be an animal model of TS.

Disclosures: **J. Du:** None. **H. Lee:** None. **L. Chiou:** None.

Poster

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National Cancer Institute P50 CA015962-02

Title: Mouse models of human PIK3CA-related segmental overgrowth have treatment-responsive epilepsy

Authors: ***A. ROY**[1], J. SKIBO[1], F. KALUME[1], J. NI[2], S. RANKIN[3], Y. LU[4], W. B. DOBYNS[1], G. B. MILLS[4], J. J. ZHAO[2], S. J. BAKER[3], K. J. MILLEN[1];
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Abstract: Phosphoinositide 3-kinases (PI3Ks) are an essential family of enzymes involved in cell signaling, regulating fundamental processes such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking; and are long studied for their roles in cancer. Mutations in the catalytic subunit of phosphoinositide 3-kinase (*PIK3CA*) have recently been associated with a wide spectrum of brain and body overgrowth phenotypes. In the brain, the phenotypic spectrum of *PIK3CA*-related segmental cortical dysplasia includes bilateral dysplastic megalencephaly, hemimegalencephaly and focal cortical dysplasia, the most common cause of intractable pediatric epilepsy. The role of these mutations in these developmental disorders, however, is not understood. Megalencephaly or "large brain" is a developmental brain overgrowth disorder, associated with intellectual disability, autism, epilepsy, hydrocephalus, cerebellar tonsillar ectopia and a host of other developmental and functional anomalies. We generated mouse models expressing the most common activating *Pik3ca* mutations (*H1047R* and *E545K*) in developing neural progenitors. These accurately recapitulate all the key pathological features including enlarged brain size, cortical malformation, hydrocephalus and epilepsy, with phenotypic severity dependent on the mutant allele and its time of activation. Underlying mechanisms include altered rate of proliferation, cell size and white matter dysplasia. Notably, we demonstrate that acute suppression of PI3K signaling in adult mice, despite the presence of dysplasia, has dramatic anti-seizure benefit. Thus PI3K inhibitors offer a promising new avenue for effective anti-epileptic therapy for intractable pediatric epilepsy patients.

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Poster

686. Developmental Disorders: Animal Models III

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 686.19/I25

Topic: C.06. Developmental Disorders

Support: INSK. Instituto Nacional de Salud Kellogg (3152)

Ciencia Basica CONACYT (1322)

Title: The iron deficiency diet during development induces oxidative stress in relation to age and gender

Authors: *P. VIEYRA-REYES[1], C. JIMENEZ-GARCES[2], M. HERNANDEZ-GONZALEZ[2], D. MILLA-ALDACO[3];
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Abstract: Iron is a trace element and structural part of antioxidant enzymes, its requirements vary according to age and gender. We hypothesized that iron deficiency (ID) leads to an increase in free radicals which mainly affect the brain, and the severity of damage would therefore be dependent on age and gender. Two groups of Wistar rats were evaluated evolutionarily: 100 rats (50 male; 50 female) with ID diet, and 100 rats (50 male; 50 female) with standard diet. Both groups were offspring from mothers who were previously under the same dietary intervention. The ages studied roughly correspond to stages of human development: birth (0 postnatal day "PND" in rats); childhood (21-PND), early-adolescence (42-PND), late-adolescence (56-PND) and adulthood (70-PND). The following biomarkers in brain, blood and liver were analyzed: lipid peroxidation products (LPO); protein carbonyl content and activity of the antioxidant enzymes; superoxide dismutase; catalase and glutathione peroxidase. It was demonstrated that ID-subjects are born with high levels of LPO in brain and low antioxidant activity, the damage being more severe in males. After birth, antioxidant defense focuses on central level (brain) in ID-females and on peripheral level (blood and liver) in ID-males. In two critical stages of development, birth and late-adolescence, antioxidant protection is insufficient to counteract oxidative damage in ID-subjects. Moreover, we observed that the variability of results in literature on oxidative stress and ID comes from gender and age of subjects under study. With this, we can establish patterns and exact moments to carry out studies or treatments.

Disclosures: P. Vieyra-Reyes: None. **C. Jimenez-Garces:** None. **M. Hernandez-Gonzalez:** None. **D. Milla-Aldaco:** None.

Poster

686. Developmental Disorders: Animal Models III

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Program#/Poster#: 686.20/I26

Topic: C.06. Developmental Disorders

Support: Indiana University Collaborative Research Grant

Indiana Institute of Biomedical Imaging Sciences Research Development Grant

Title: Neurological consequences of a TMEM67 mutation in the Wpk rat model of hydrocephalus

Authors: J. S. PETERS[1], *J. A. MEYER[1], J. W. SHIM[4], E. MAUE[4], S. AHMED[4], J. C. WATSON[2], L. JIANG[1], A. A. RILEY[1], B. P. MCCARTHY[1], S. A. PERSOHN[1], D. H. FULKERSON[3], P. R. TERRITO[1], B. L. BLAZER-YOST[2,4];
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Abstract: Ciliopathies are a genetic disorder of the cellular cilia that manifest as a spectrum of pathologies including respiratory, renal, cochlear, retinal, and brain aberrations. Primary cilia function as mechano- or chemo-receptors and stimulate intracellular signaling that preserve homeostatic ion and water transport such as those involved in cerebrospinal fluid production and resorption. Hydrocephalus has been described in both animal models and humans suffering from ciliopathies. The Wistar polycystic kidney (Wpk) rat is orthologous to the human Meckel-Gruber Syndrome and exhibits many of the characteristics of pediatric hydrocephalus. A point mutation in trans-membrane protein 67 (TMEM67) leads to elongated cilia on the choroid plexus, and increased fluid accumulation in the ventricles. Based on this, the purpose of this work is to characterize the anatomical and functional consequences of this point mutation in developing rats. Genotyping has discerned that littermates displaying severe hydrocephalus are homozygous recessive (TMEM67-/-) while those possessing TMEM67+/- heterozygosity display mild hydrocephalus from early postnatal periods through adulthood. Head dimensions of the TMEM67-/- animals were significantly increased over the control animals when measured in vertical ($p=0.006$) or horizontal orientation ($p=0.007$). T2 weighted MRI scans of ventricular volumes confirmed the individual genotypes were statistically different ($p<0.001$), where lateral ventricle volumes for wild type, TMEM67+/- and TMEM67-/- were 1.5±0.6, 6.7±1.8, and 71.9±9.2µl at P7-8, and 2.6±0.3, 16.7±5.4, and 491.2±61.9µl at P17-18, respectively. To determine potential alteration in histo-architecture, and using the pineal gland as reference, we found changes in TMEM67+/- and TMEM67-/- that had significant alteration of visual cortex and midbrain reticular formation. To confirm the histo-architecture effect of hydrocephalus on cerebral glucose utilization, 2-deoxyglucose uptake was examined in P17-18 rats, where isotope concentrations were normalized to cerebellar cortex in order to allow for comparisons between wild type and hydrocephalic (TMEM67+/-, TMEM67-/-) groups. By this method, glucose uptake values were statistically lower with brain region ($p<0.0001$) and genotype ($p<0.0001$), and are in agreement with previous reports of hydrocephalus models. Based on these findings, the Wpk rat model provides a unique platform to study the development of both severe and mild hydrocephalus. Moreover, this rodent model provides an opportunity to screen for potential drug candidates that may be useful in treating human forms of hydrocephalus.

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Poster

686. Developmental Disorders: Animal Models III

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 686.21/I27

Topic: C.06. Developmental Disorders

Support: Swiss National Science Foundation Grant n 310030_146217

Title: Genome-wide methylation changes following early and late prenatal immune activation: focus on the prefrontal cortex

Authors: ***J. RICHETTO**[1], R. MASSART[2,4], M. SZYF[3], M. A. RIVA[5], U. MEYER[1];
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Abstract: Objective: Epidemiological and experimental evidences demonstrate that maternal exposure to infection during gestation is an environmental risk factor for a variety neurodevelopmental disorders, such as schizophrenia and autism. Moreover, the precise timing of the prenatal infection seems to be critical, as it can determine specific clusters of behavioral and morphological phenotypes in the offspring. Thus, we used a specific animal model of prenatal immune challenge to investigate the possible molecular mechanisms underlying differences and similarities in specific clusters of behavioral abnormalities brought on by infection at two different time points in pregnancy. First of all, we compared adult behavioral features in offspring born to mothers subjected to infection early or late during gestation. Secondly, we investigated the possible molecular mechanisms underlying such abnormalities, focusing our attention on how prenatal infection could affect DNA methylation patterns in the prefrontal cortex. Methods: C57BL/6 mice were treated with the synthetic viral mimetic poly(I:C) (5 mg/kg, i.v.) or control (saline, i.v.) solution on gestation day 9 or on gestation day 17. Offspring were subjected to cognitive and behavioral testing in adulthood, and then capture-sequencing DNA methylation analysis and subsequent q-PCR validation were performed on the

prefrontal cortex. Results: Prenatal exposure to Poly(I:C) led to a variety of behavioral abnormalities, some of which were common to both time points, while others, as for example prepulse inhibition, were specific to one time point. Specific and overlapping changes were also observed when considering the DNA methylation analysis in the prefrontal cortex. In particular, both early and late exposure to prenatal infection led to extensive changes in DNA methylation, and a significant portion of these differentially methylated regions is common to both time points of infection. Different genes, such as Mid1, Ntm and Nrxn2, stood out as top candidates for deeper investigation based on their involvement in neurodevelopment and psychiatric disorders. Conclusions: Our results provide new insight into the molecular mechanisms mediating the association between prenatal infection and adult vulnerability to psychiatric disorders, and, more specifically, provide further knowledge regarding the impact of the precise timing of the infection. Moreover, our results uncover possible targets of future studies and point to a possible role of DNA methylation in mediating the detrimental effects of prenatal immune challenge, consistent with similar reports in human studies.

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Poster

686. Developmental Disorders: Animal Models III

Location: Hall A

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Program#/Poster#: 686.22/I28

Topic: C.06. Developmental Disorders

Title: Decreased performance in an active avoidance task in the valproic acid rat model of autism

Authors: ***J. R. HOLLERMAN**[1], T. J. BARR[2], W. Z. PADEN[1], J. D. CROSS[1];
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Abstract: The initial proposal for the use of prenatal exposure of rats to valproic acid (VPA) as an animal model of autism (Rodier et al., 1997) is supported by observations of co-incidence of autistic symptoms and fetal Valproate Syndrome in humans (e.g., Christianson et al., 1994; Williams and Hersch, 1997), as well as behavioral studies using the model (e.g., Schneider and Przewlocki, 2005; Schneider et al., 2008). Anatomically, the amygdala has received a great deal of attention both in human studies (e.g., Bechevalier and Loveland, 2006) and in the VPA model, where electrophysiological effects have also been characterized (Markram et al., 2008). The further functional implications of the effects of prenatal VPA on the amygdala are not clear. In

order to help further elucidate this, VPA-exposed rats were tested for preference for familiarity to verify the "autistic" phenotype, as well as performance on an active avoidance task. VPA exposure consisted of administration of VPA (470mg/kg, i.p.) to dams at gestational day 12.5. Rats were raised by their dams and allowed to grow to adulthood (8 weeks). Male VPA-exposed (n = 12) and control (n= 8) rats were then tested for relative preference for interacting with a novel versus a familiar object and trained on a shuttle box, active avoidance task using a 5sec 2.4kHz tone as CS and 0.1mA electric foot shock (max 5sec duration) as the aversive stimulus. Rats were trained on 50 trials per day over 5 days with performance assessed in terms of escape and avoidance responses (individually and combined). Object preference was calculated as (time spent with a novel object - time spent with familiar object)/ (time spent with a novel object + time spent with familiar object), producing negative values for novelty preference and positive values for familiarity preference. While controls expressed an expected preference for novelty (M = -0.28, SEM = 0.06), VPA-exposed rats differed significantly, displaying a preference for familiarity (M = +0.25, SEM = 0.05). In the acquisition of the shuttle box avoidance task, performance of the VPA-exposed rats differed significantly from that of control rats. VPA rats made fewer escapes, avoidances, and total responses than Controls across all 5 days. However, the two groups showed similar rates of acquisition, with both groups displaying an increase in avoidance responses (and total responses) across the 5 days. While it is not yet clear whether this difference reflects a specific learning deficit or is due to a deficit in nociceptive processing, it does indicate a behavioral effect on a putatively amygdala-dependent learning task in the VPA model.

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Poster

686. Developmental Disorders: Animal Models III

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 686.23/I29

Topic: C.06. Developmental Disorders

Support: H. Lundbeck A/S

Danish Agency for Science Technology and Innovation

Title: Behavioral and transcriptomic alterations in 15q13.3 homozygous knockout mice

Authors: ***A. FORSINGDAL**[1,2], M. BERTALAN[2], K. FEJGIN[1], V. NIELSEN[1], T. WERGE[2], J. NIELSEN[1];

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Abstract: Genome wide association studies have revealed that certain copy number variants (CNVs) strongly increase the risk of schizophrenia and other psychiatric diseases. One such CNV is a 1.5 MB long hemizygous deletion located in the 15q13.3 region that covers 6 genes (FAN1, MTMR10, TRPM1, KLF13, OTUD7A, and CHRNA7). The 15q13.3 microdeletion increases the risk of schizophrenia, epilepsy and autism (Malhotra and Sebat, 2012). A mouse model of the human 15q13.3 hemizygous microdeletion syndrome has been generated. Characterization of the model identified disease-related phenotypes (Fejgin et al., 2013). However, the mechanisms underlying increased disease risk in the 15q13.3 microdeletion syndromes remains unknown. Human cases of homozygous microdeletion carriers have also been reported, all with severe impairments (Hoppman-Chaney et al., 2013). We hypothesized that 15q13.3 homozygous knockout mice would display stronger phenotypes than 15q13.3 hemizygous mice and thereby facilitate mechanistic studies. 15q13.3 knockout mice were characterized by basic physiological and behavioral tests as well as disease related behavioral tests. Indeed, 15q13.3 knockout mice display stronger disease-related phenotypes than the 15q13.3 hemizygous deletion mice. In order to elucidate the underlying biological mechanisms of these disease-related phenotypes, we have initiated transcriptomic studies of the 15q13.3 homozygous knockout mice. A number of genes are differentially expressed in 15q13.3 homozygous knockout mice compared to wildtype mice. The expression profile of these mice provides cues to which biological mechanisms that predispose 15q13.3 deletion carriers to psychiatric diseases and guides future mechanistic exploration.

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Poster

686. Developmental Disorders: Animal Models III

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 686.24/I30

Topic: C.06. Developmental Disorders

Support: South African Medical Research Council

South African National Research Foundation

Title: Effect of cocaine on striatal dopamine clearance in an animal model of developmental stress and attention-deficit/hyperactivity disorder

Authors: *V. A. RUSSELL[1], J. S. WOMERSLEY[2], L. A. KELLAWAY[2], D. J. STEIN[3], G. A. GERHARDT[4];
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Abstract: Attention-deficit/hyperactivity disorder (ADHD) and developmental stress are considered risk factors for the development of drug abuse and all 3 processes share underlying disturbances in dopamine neurotransmission. The dopamine transporter (DAT) is responsible for the rapid reuptake of dopamine and thereby carefully controls the extra-synaptic concentration of dopamine. Inhibition of DAT by psychostimulants (e.g. cocaine) increases the extracellular concentration of dopamine and is responsible for the rewarding properties of drugs. Though research has indicated that a history of developmental stress and a diagnosis of ADHD increase the likelihood of developing drug abuse, the physiological mechanism underlying this relationship is not fully understood. In the current study, the effect of maternal separation (MS), a model of developmental stress, on striatal dopamine clearance before and after cocaine administration was investigated using the spontaneously hypertensive rat (SHR), a well-validated model of ADHD. *In vivo* chronoamperometric recordings revealed that there was no difference between SHR and the control Wistar-Kyoto (WKY) strain in the cocaine-induced elevation of dopamine levels. However, the cocaine-induced increase in time to clear 50% of exogenously applied dopamine was elevated in maternally separated SHR compared to non-separated SHR but had no effect in WKY. These findings suggest that a strain x environment interaction altered DAT function, prolonging elevated levels of dopamine and thereby potentially increasing the rewarding properties of this drug in SHR.

Disclosures: V.A. Russell: None. **J.S. Womersley:** None. **L.A. Kellaway:** None. **D.J. Stein:** None. **G.A. Gerhardt:** None.

Poster

686. Developmental Disorders: Animal Models III

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Program#/Poster#: 686.25/I31

Topic: C.06. Developmental Disorders

Title: Social isolation during the critical period affects excitatory neuronal activity in mouse medial prefrntal cortex

Authors: *K. YAMAMURO[1], H. YOSHINO[1], Y. OGAWA[2], K. OKAMURA[1], Y. NISHIHATA[1], Y. SAITO[2], T. KISHIMOTO[1];
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Abstract: Social experience is crucial for the development of forebrain function and the maturation of medial prefrontal cortex (mPFC). Rearing mice in post-weaning social isolation produces prefrontal dysfunction and poor social interaction with hypomyelination in mPFC (Makinodan et al. Science 2012). However it isn't well-known about the alteration in neural circuits of mPFC induced by social isolation. In the present study, we examined the change of excitatory synaptic inputs and membrane properties of layer 5 pyramidal cells in mouse mPFC induced by social isolation, with whole-cell patch clamp recording in the brain slices. After weaning at P21, the 4 male littermates were randomly divided into one isolate-housing mouse and three group-housing mice. After the isolation period (from P21 to P35), the isolated mouse was again reared together with his three grouped littermates. We also examined the change induced by isolate-housing during the later period (from P35 to P49). The recording from each mouse was done at P63-69. We found that the spontaneous excitatory postsynaptic current (sEPSC) frequency and miniature excitatory postsynaptic current (mEPSC) frequency were significantly lower in P21-P35 isolated mice than in grouped mice. On the other hand, membrane properties of layer 5 pyramidal cells were not changed in P21-P35 isolated mice. And there was no significant difference in sEPSC and mEPSC frequency between P35-P49 isolate-housing mice and group-housing mice. Moreover, in somatosensory cortex, there was no significant difference in sEPSC and mEPSC frequency between P21-P35 isolated mice and grouped mice. These results show that only 2 weeks social isolation (from P21 to 35) exclusively reduces excitatory synaptic inputs on layer 5 pyramidal cells of mouse mPFC and suggest that social experience during the critical period is pivotal in the development of mPFC excitatory neural circuit.

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Poster

686. Developmental Disorders: Animal Models III

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Topic: C.06. Developmental Disorders

Support: Japan Foundation for Neuroscience and Mental Health

Title: Docosahexaenoic acid (DHA) rescued autistic symptoms accompanied by dopaminergic change on a gene/prenatal stress mouse model

Authors: *F. MATSUI[1,2], K. YOSHIMOTO[3], P. HECHT[2], K. FRITSCHE[2], M. WILL[2], D. BEVERSDORF[2];
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Abstract: Autism Spectrum Disorder (ASD) is characterized by impairments in social interaction and social communication, and repetitive and stereotyped behaviors. While genetics is thought to play a large role in the disorder, environmental factors, such as prenatal stress are also thought to contribute to it. We previously reported that prenatal stress exposure in stress-susceptible heterozygous serotonin transporter (SERT) KO pregnant dams in a mouse model altered social interaction and repetitive behavior in the male offspring (SERT/stress mice), and additionally found that docosahexaenoic acid (DHA) rescued the autism associated symptoms. DHA plays an important role in the functioning and development of the central nervous system, but the mechanism how DHA may impact the development of autism in this setting is unknown. In the present study, we measured monoamine levels in selected brain regions in the SERT/stress mouse model. Then, we measured monoamine levels in three treatment groups; DHA rich diet continuously from breeding, DHA rich diet only after weaning, and control diet only. The dopamine (DA) content in the striatum was significantly increased in the SERT/stress mice compared with wild type (WT) mice. Noradrenaline and serotonin content were not changed between the SERT/stress and WT mice in the selected brain regions. Moreover, DA content in the striatum was significantly reduced in the SERT/stress mice with the DHA rich diet provided continuously from breeding. The results indicate that autism-associated behavior in this setting can be reversed with DHA accompanied by changes in the dopaminergic system. Future research will be necessary to explore whether DHA treatment during pregnancy may be important for rescuing the symptoms associated with some etiologies of ASD in the clinical setting.

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Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 687.01/I33

Topic: C.08. Ischemia

Support: NIH grant R01AT007429

NIH grant R01NS046400

Title: Novel usefulness of cylinder test for assessing long-term dysfunction in mouse model of mild ischemic stroke

Authors: *L. LIU[1], M. WEIDER[1], Y. DWEIK[1], S. DORE[1,2];
[1]Anesthesiology, Ctr. for Translational Res. in Neurodegenerative Dis., [2]Departments of Neurology, Psychiatry, Pharmaceutics, Psychology, and Neurosci., Univ. of Florida, Col. of Med., Gainesville, FL

Abstract: Stroke is a debilitating neurological disorder, which is more disabling than it is fatal-remains one of the leading causes of disability. Permanent focal middle cerebral artery occlusion (pMCAO) C57BL/6 mouse model is believed to be one of the most predictable and potentially useful stroke translational models. Long-term sensorimotor function evaluation is a key component for testing the efficacy of potential therapeutics. However, till now few behavior tests provide reliable functional outcome evaluation after stroke. Therefore, there is an important need to develop sensitive and reliable behavior tests. In this study, we investigated the sensorimotor functional deficit and recovery in pMCAO model mice over 4 weeks by using a behavior test battery including locomotor activity, cylinder test and corner test. In comparison to the only parameter-total rate of forelimb use in traditional cylinder test, we first used multiple parameters to characterize the sensorimotor asymmetry, including the rates of first use of forelimb (single and both) in rearing instances with contacting the cylinder wall. The novel use and analytic approach in cylinder test provide more sensitive and reliable assessment to detect the sensorimotor deficit. Thus, this novel use of cylinder test will be most instrumental and promising in investigating therapy strategy of stroke.

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Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

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Program#/Poster#: 687.02/I34

Topic: C.08. Ischemia

Title: Effects of transient ischemia and D-cycloserine on aromatase expression in the rat brain

Authors: *J. DHAWAN, A. BIEGON;
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Abstract: Estrogen is thought to be neuroprotective in animal models of stroke and in this context, transient increases in the levels of the estrogen synthesizing enzyme aromatase were considered to represent activation of an endogenous neuroprotective mechanism. We have used quantitative immunohistochemistry to explore the long term effects of stroke and the partial NMDA receptor agonist D-Cycloserine (DCS) on aromatase expression in cortex and striatum of rats subjected to transient (90 min) focal ischemia through occlusion of the middle cerebral artery (MCAO). Rats were given i.p injections of DCS (10 mg/kg, N=14) or vehicle (PBS, N=14) 24 hours post reperfusion. Eight rats subjected to sham surgery served as controls. Animals were killed more than 4 weeks after MCAO, following repeated evaluations for neurological deficits and memory. Brains were quickly frozen and coronal sections at the level of the infarct were incubated with a polyclonal antibody to aromatase and stain developed with 3,3-diaminobenzidine (DAB). Sections were then counterstained with hematoxylin. Staining density was analyzed in striatum and cortex using ImageJ (NIH) software at low magnification. The number of aromatase positive neuronal cells in layers II to VI of the frontal were counted with ImageJ using stereological principles at 200x magnification. Increased expression of aromatase relative to controls was still evident in MCAO rats more than 4 weeks after occlusion, in the peri infarct zone but also in the cortex; however aromatase density in DCS treated rats was significantly lower than in vehicle treated animals. Analysis of aromatase positive neurons in the frontal cortex revealed layer- and treatment related effects. MCAO animals had a significantly larger number of aromatase positive neurons in layers II ,IV and VI relative to controls. However, the effects were significantly smaller in DCS treated animals. These results show that DCS, previously shown to improve neurological outcome, memory function and BDNF expression in rat models of brain injury and stroke; can also reverse, at least in part, a persistent increase in aromatase expression in cortical neurons induced by transient focal ischemia.

Disclosures: J. Dhawan: None. **A. Biegon:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Program#/Poster#: 687.03/I35

Topic: C.08. Ischemia

Support: UNSW Australia Gold star award

Title: Neuronal remodelling following cerebral photothrombotic infarcts in mice

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Abstract: Rodent models of stroke typically employ middle cerebral artery occlusion to induce ischemic brain injury. This method produces lesion volumes that have considerable variation and typically encompass a large portion of the forebrain, resulting in a high mortality rate. These factors make study of neuroprotection problematic. An alternative model for focal ischaemic brain injury utilizes photothrombotic lesions. Despite the emerging prominence of this stroke model, there is a paucity of information on the spatiotemporal injury sequelae in mice that we address here. Platelet aggregation was triggered by photosensitising the brain microvessel endothelium, via a tail vein injection of rose bengal dye, followed immediately by irradiation of the target brain surface (midline; 2 mm rostral to lambda) with green light (2 mm diameter, 532 nm, ~ 3 mW, 6 min) under isoflurane anaesthesia. Mice were euthanized and their brains removed 2 hours, 1, 4, 7 or 14 days post injury (DPI; n = 3 for each time point). Brains were initially stained with 2, 3, 5- triphenyltetrazolium chloride to visualize viable tissue. The tissue was then fixed in 4% paraformaldehyde and cryosectioned (50 µm). The infarct volume and ischemic tissue remodelling were assayed using dark field imaging and haematoxylin and eosin (H & E) staining. Under dark field imaging injury-associated oedema and tissue structure changes manifest as increased tissue opacity. The infarct volume, calculated from the dark field images, increased up to 4 DPI. H&E assessment showed systematic transition of histopathology over time. At 2 hours post injury, neuronal loss was observed with microglial infiltration at the site of lesion. By 1 DPI there was a complete loss of neurons in the infarct core. Consistent with the dark field images, there was extensive lateral expansion of the lesion observed 4 DPI, along with loss of neuronal cytoplasm and evidence of extravasation. Sections prepared from mice 7 DPI displayed less extravasation and greater microglial infiltration than those prepared 4 DPI. Further microglial invasion into the site of lesion was observed 14 DPI, delineating the infarct boundary distinctly. The spatiotemporal changes in cell composition within the ischemic core and surrounding penumbra suggest the remodelling of cerebral tissue, with darkfield imaging confirming the reduction in oedema at 7 DPI onwards. The 100 % survival rate, consistency in lesion volume and cell response profile over time in this study validate this preclinical stoke model as a neuroprotective treatment assay. The protocol was approved by the UNSW Australia Animal Care and Ethics Committee.

Disclosures: **N. Gorlamandala:** None. **J. Parmar:** None. **A.J. Craig:** None. **J.M. Power:** None. **A.V. Krishnan:** None. **G.D. Housley:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 687.04/I36

Topic: C.08. Ischemia

Support: NIH Grant NS046400

Title: Laropiprant, a clinically tested prostaglandin D2 DP1 receptor antagonist, minimizes brain injury following intracerebral hemorrhage

Authors: M. MENDES[1,2], S. DORE[1,2,3], *A. S. AHMAD[1,2];
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Abstract: Laropiprant, a clinically tested prostaglandin D_2 DP1 receptor antagonist, minimizes brain injury following intracerebral hemorrhage Monique Mendes[1,2], Sylvain Dore[1,2,3], Abdullah Shafique Ahmad[1,2] [1]Department of Anesthesiology, [2]Center for Translational Research in Neurodegenerative Disease, [3]Departments of Neurology, Psychiatry, Pharmaceutics, Psychiatry, and Neuroscience, University of Florida, Gainesville, FL The respective importance of PGD2 and its receptor DP1 in the vasculature, the blood, and the brain warrants further examination of their role in intracerebral hemorrhage. In this study, we tested whether deletion of DP1 receptor or its blockade by the selective antagonist Laropiprant improves functional and anatomical outcomes following ICH by limiting intracranial bleeding. Wildtype (WT) and DP1-/- C57BL/6 mice were subjected to ICH by giving a single dose of collagenase in the striatum. Neurologic deficits, brain injury volume, and edema volume were calculated at 72h. In another set of cohorts, WT mice were subjected to ICH followed by single i.p. injection of Laropiprant. To test whether the beneficial effects of DP1 receptor inhibition could be through reduced intracranial bleeding, non-ICH WT and DP1-/- mice were tested for tail bleeding time following the Laropiprant treatment. To further test the effect of Laropiprant on *ex-vivo* coagulation, mouse blood was mixed with vehicle or Laropiprant and the uncoagulated content was quantified. Finally, the role of DP1 inhibition in modulation gliosis was also tested by performing immunoreactivity for Iba1 and GFAP on the brain sections of WT and DP1-/- mice. A significant decrease in the injury volume (4.8±1.7 vs 14.1±4.5mm3; $p<0.001$) and edema volume (4.5±1.8 vs 13.2±4.1mm3; $p<0.001$) in DP1-/- was observed. Furthermore, WT mice treated with Laropiprant also exhibited significantly lower brain damage as compared with the vehicle treated group (6.2±2.2 vs 12.7±5.1mm3; $p<0.01$). Interestingly, the tail bleeding time was also

significantly lower in Laropiprant group (219.4±75.2sec; $p<0.001$) as compared with the vehicle group (345.2±45.6sec), and similarly the uncoagulated content was also lower in Laropiprant group (356.2±53.6µL; $p<0.001$) as compared with the vehicle group (486.4±26.7µL). Interestingly, a significant decrease in Iba1 immunoreactivity and increase in GFAP immunoreactivity was observed in DP1-/-. Together, the data suggests that inhibition of the DP1 receptor after ICH attenuates functional and anatomical deficits partially by minimizing intracranial bleeding and neuroinflammation. [Supported by NIH R01 (SD) funds]

Disclosures: **M. Mendes:** None. **S. Dore:** None. **A.S. Ahmad:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Program#/Poster#: 687.05/I37

Topic: C.08. Ischemia

Title: Intermittent surge of brain-heart coupling prior to sudden death in ischemic rats

Authors: ***F. TIAN**[1], D. LI[1], T. LIU[1], M. WANG[1,2], J. BORJIGIN[1];
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Abstract: Sudden death is an important but under-recognized consequence of stroke. Acute stroke can disturb central control of autonomic function, result in cardiac dysfunction, and ultimately lead to sudden death. Previous study showed that bilateral common carotid artery (BCCA) ligation in spontaneously hypertensive stroke-prone rats (SHRSP) could lead to sudden death. However, the underlying mechanism by which cerebral ischemia induces cardiac dysfunction and sudden death is not well understood. The objective of this study is to investigate the functional interactions between the brain and the heart during this process using advanced signal processing techniques. ECoG (from 6 cortical loci) and ECG signals were simultaneously collected from SHRSP and control Wistar Kyoto (WKY) rats before and following BCCA ligation. Consistent with published studies, BCCA ligation resulted in 100% mortality in SHRSP rats within 24 hours, whereas no mortality was observed in WKY rats. In contrast to WKY rats, SHRSP rats exhibited intermittent and elevated cardiac event-related potentials in the cortex and increased brain-heart coherence following the onset of ischemia. SHRSP rats also exhibited reduced heart-rate variability prior to sudden death. Further studies are aimed at investigating the effective connectivity between the brain and the heart during ischemia. This study is expected to improve our understanding of the mechanism of stroke-induced cardiovascular abnormalities and

sudden death, and may provide important information for future treatment of cardiac dysfunction and prevention of sudden death after ischemic stroke.

Disclosures: **F. Tian:** None. **D. Li:** None. **T. Liu:** None. **M. Wang:** None. **J. Borjigin:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Topic: C.08. Ischemia

Support: NIH Grant P20GM109098

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NIH Grant T32GM081741

Title: Post-stroke spontaneous hypothermia predicts stroke severity and is correlated with mitochondrial impairment

Authors: ***X. REN**[1], H. HU[1], D. DOLL[2], J. SUN[1], J. WIMSATT[3], M. KESSLER[4], J. SIMPKINS[1];
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Abstract: Stroke is the second leading cause of death and the leading cause of disability worldwide. Animal models of ischemic stroke are indispensable tools to investigate the pathophysiological mechanisms involved in ischemic brain injury and to develop novel therapies for stroke. Controlled-hypothermia is known to protect ischemic outcomes in human subjects and experimental animal studies in stroke. However, spontaneous hypothermia has confounded experimental results and pharmacological studies in stroke, and the mechanisms are poorly understood. In this study, we performed a correlation and regression analysis of the pooled data (n=311) using mouse models of transient and permanent occlusion of the middle cerebral artery and determined a relationship between post-stroke body temperature and stroke severity in experimental stroke. We found that stroke mice had lower body temperature compared to sham mice within 6 hours post-surgery. Rectal temperature within 6 hours post-stroke was

significantly negatively correlated with stroke outcomes. Linear regression with neurological deficits as the dependent variable showed that low body temperature was significantly associated with high neurological score at 6-, 24-, and 48-hour end points ($P<0.0001$), and larger infarct volumes in cortex ($P<0.0001$), striatum ($P=0.0013$) and total hemisphere ($P<0.0001$). Lipopolysaccharide (LPS) has been recently reported to inhibit mitochondria in cerebrovascular endothelial cells, and the LPS-cohort analysis indicated rectal temperature at 6 hours post-stroke had strong negative correlations with neurological deficits ($r=-0.6564$, $P<0.0001$), infarct volumes in cortex ($r=-0.6976$, $P=0.0055$), and total hemisphere ($r=-0.5992$, $P=0.0235$). Rotenone (mitochondrial respiratory chain complex I inhibitor) cohort analysis indicated rectal temperature at 6 hours post-stroke had very strong negative associations with neurological deficits ($r=-0.7452$, $P<0.0001$), infarct volumes in cortex ($r=-0.7368$, $P=0.0011$) and total hemisphere ($r=-0.8231$, $P<0.0001$), and strong negative association with stratum infarction ($r=-0.6633$, $P=0.0051$). We concluded that post-stroke spontaneous hypothermia predicts stroke severity and mitochondria may play a pivotal role in this hypothermic response in acute stroke.

Disclosures: **X. Ren:** None. **H. Hu:** None. **D. Doll:** None. **J. Sun:** None. **J. Wimsatt:** None. **M. Kessler:** None. **J. Simpkins:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Topic: C.08. Ischemia

Support: Loyola Research Institute

Department of Veterans Affairs

Title: An examination of sex differences in neocortical excitatory synapse number in the aged rat

Authors: ***V. BORKOWSKI**[1,2,4], S.-Y. TSAI[4], J. L. MARTIN[5], H. HIOKI[6], A. E. MARINOPOULOS[4], K. S. HSU[4], C. M. PAPADAPOULOS[4], G. L. KARTJE[4,3];
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Abstract: Sex-based differences in recovery from stroke are an area of research not yet fully explored, especially with regard to aged rats performing sensorimotor tasks, which model human post-stroke functional recovery. In the human clinical population, post-menopausal females have been shown to recover from stroke less successfully than age-matched males, and therefore studying sex differences in rat models of stroke recovery is clinically relevant. We therefore set out to determine if differences in excitatory synaptic number were evident in different sexes, which may influence recovery from stroke. 18 month-old Fischer 344 male and female aged rats were used for these studies. Rats were divided into three groups: ovariectomized (OVX) females, intact females, and age-matched males. Rats were trained in the skilled forelimb reaching task, then received adeno-associated virus (AAV8.cmv.myrGFP-LDLRct) injections to the motor cortex to pre-label cortico-efferent pyramidal neurons in layer V. At the end of three or eight weeks of behavioral testing, rats were sacrificed for immunohistochemistry to quantify excitatory synapses in pyramidal layer V forelimb motor cortex. Neurons that were GFP-positive with a typical pyramidal shape and good dendritic labeling were identified via confocal microscopy. Using 63x oil magnification, basilar dendrites were examined for presynaptic (vGlut1 or vGlut2) markers and co-localization with the postsynaptic marker NMDAR-NR1. The number of co-localized synapses was quantified using an orthogonal Z-stack (Z step .89um, 16 Z-steps) on basilar dendrites for a 40um length starting at the cell soma. Our preliminary results showed that after 3 weeks of behavioral testing, no differences were found between groups in the number of excitatory synapses on layer V pyramidal neurons. However, after 8 weeks of testing, the number of excitatory synapses in the aged OVX female group was significantly less than the intact females and age matched males. These results indicate a possible long-term effect of estradiol depletion on neocortical synaptic number in aged females. Further work is in progress to correlate these findings with post-stroke recovery in the aged rat.

Disclosures: **V. Borkowski:** None. **S. Tsai:** None. **J.L. Martin:** None. **H. Hioki:** None. **A.E. Marinopoulos:** None. **K.S. Hsu:** None. **C.M. Papadapoulos:** None. **G.L. Kartje:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Program#/Poster#: 687.08/I40

Topic: C.08. Ischemia

Support: NIH 5R01HD061363

Title: Classification of the lateral cerebellar nucleus neuronal activity during skilled reaching tasks

Authors: *H.-J. PARK[1], J. COOPERRIDER[1], H. H. CHAN[1], C. WATHEN[1], J. T. GALE[1], M. D. JOHNSON[2], K. B. BAKER[2], A. G. MACHADO[1];
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Abstract: Stimulation of the lateral cerebellar nucleus (LCN) has been shown to be effective for post-stroke rehabilitation in a model of rodent focal ischemia, likely through activation of broad motor cortical areas, including the perilesional cortex, via the excitatory di-synaptic dentato-thalamo-cortical (DTC) pathway. In our previous studies, deep brain stimulation (DBS) of the LCN was conducted in a pre-programmed, open-loop fashion. In contrast to open-loop stimulation, closed-loop stimulation may improve post-stroke rehabilitation by selectively stimulating or adaptively changing the stimulation parameters of LCN DBS based on the brain state. Electrophysiological recordings of the LCN during a behavioral task may assist in estimating the brain states to be utilized for closed-loop stimulation. For selective stimulation with stimulation parameter adaptation, estimation and classification of the brain states using machine learning algorithms are required with respect to the important event markers in a skilled reaching behavioral task. In order to record event related modulation of brain activity, multiple contact microelectrode arrays was placed in the deep cerebellum of the rats. The rats were trained on a skilled pellet-reaching task with the paw ipsilateral to the implanted electrode. The performance of the reaching task was videotaped to classify trials into successful or unsuccessful, and electrophysiological recordings were synchronized with the behavioral data using an infrared beam, which detected the reaching motion. The electrophysiological recordings were wirelessly transmitted from the headstage to the receiver, and the data were analyzed offline. Time-frequency analysis on the filtered local field potentials (LFP) and/or neuronal spike activity analysis generated features for the classification. Different classifiers were tested in order to build an optimal classifier, including a generalized linear model and support vector machine.

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Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 687.09/I41

Topic: C.08. Ischemia

Title: Granulocyte-colony stimulating factor enhances the angiogenetic effect of indirect bypass surgery for chronic cerebral hypoperfusion in a rat model

Authors: *K. ORITO, M. MORIOKA;
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Abstract: OBJECTIVE - Granulocyte-colony stimulating factor (G-CSF) mobilizes hematopoietic bone marrow cells into systemic circulation and has been used clinically to treat chemotherapy-induced neutropenia. Recently, G-CSF has been shown to have neuroprotective and angiogenetic effects in acute cerebral infarction. We hypothesized that G-CSF could act as an enhancer of angiogenesis after indirect bypass surgery. METHODS - Chronic cerebral hypoperfusions were induced in male Wistar rats by permanent bilateral internal carotid artery occlusion (BICAO). After BICAO, unilateral indirect bypass and encephalo-galeo-synangiosis (EGS) were performed and human recombinant G-CSF (10 μg/kg) or saline was injected intramuscularly for 5 consecutive days. We measured regional cerebral blood flow (rCBF) by laser Doppler flowmetry and performed immunohistochemical analysis 21 days after BICAO. RESULTS - BICAO decreased rCBF to 62.52% ± 5.8% of control ($P < 0.01$). The rCBF increased significantly 21 days after BICAO in all treatment groups ($n = 10$; $P < 0.05$) except in the G-E- group. The rCBF increase observed in the G+E+ group was significantly higher than that observed in other groups. Both G-CSF and EGS treatments significantly increased the number of small vessels ($P < 0.01$), and G-CSF and EGS showed additive effect in increasing the number of small vessels. CONCLUSION - Combined use of G-CSF and indirect bypass surgery induces an increase in rCBF and angiogenesis under cerebral chronic hypoperfusion conditions. This is the first report to demonstrate that G-CSF can enhance angiogenesis induced by indirect bypass surgery, and this combined therapy is safe and easy method of treatment.

Disclosures: K. Orito: None. **M. Morioka:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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DOD TATRC W81XWH-11-1-0634

James and Esther King Foundation for Biomedical Research Program 1KG01-33966

Title: An eye-opener for stroke: pathological consequences of reduced blood flow in the ipsilateral eye following transient middle cerebral artery occlusion in adult rats

Authors: *C. BORLONGAN[1], J.-Y. LEE[2], N. TAJIRI[3], S. ACOSTA[2], H. NGUYEN[2]; [2]Neurosurg. and Brain Repair, [3]Sch. of Physical Therapy, [1]Univ. of South Florida, Tampa, FL

Abstract: Middle cerebral artery occlusion (MCAO) in rodent remains a widely used model of ischemic stroke. Recently, many studies have reported the occurrence of retinal ischemia in animals subjected to MCAO, owing in part to the circulatory juxtaposition of the ophthalmic artery to the MCA. In this study, we employed the laser Doppler to evaluate the hemodynamic in the eye of MCAO-induced stroke rats. Brain and retinal perfusion was evaluated by laser Doppler at baseline (prior to MCAO), during MCAO, and after MCAO. Retinal function-relevant behavioral and histological outcomes were performed at 3 days post-MCAO. We also studied changing density of retinal ganglion cell and measure of optic nerve depth. Laser Doppler revealed typical reduction in brain perfusion in the ipsilateral front parietal cortical area of at least 80% reduction during MCAO compared to baseline, which returned to near baseline levels after MCAO. A significant defect in the retinal perfusion in the ipsilateral eye was detected with at least 30% reduction in perfusion during MCAO compared to baseline, which was restored to near baseline levels after MCAO. Behavioral performance in light stimulus-mediated place preference was significantly impaired in MCAO rats compared to control animals. Moreover, retinal ganglion cell density and optic nerve depth were significantly decreased in ipsilateral eye. Retinal perfusion defects closely parallel the timing of cerebral blood flow alterations in the acute stages of MCAO in adult rats. Behavioral and histological impairments further reveal the contribution of retinal dysfunction in stroke outcomes. In view of some stroke patients presenting with visual deficits, closely monitoring of brain and retinal perfusion via laser Doppler measurements may facilitate the diagnosis of stroke onset and progression, as well as treatment of stroke patients with retinal dysfunctions.

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Poster

687. Ischemia and Hemorrhage: Animal Models

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NIH Grant R01NS078223

Title: High throughput identification and quantification of peri-infarct depolarization in mouse cortex with optical intrinsic signal imaging

Authors: *J. BUMSTEAD[1], A. KRAFT[2], X. YANG[2], A. BAUER[3], J. CULVER[3], J. LEE[2], J. LU[4];
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Abstract: Objective: Focal brain ischemia results in repetitive waves of electrophysiological hyperactivity followed by silence in surrounding cortex, a phenomenon known as peri-infarct depolarization (PID). Despite the potential role that PIDs play in ischemic injury, their propagation over the entire mouse cortex has not been well studied. We developed an optical intrinsic signal (OIS) imaging system and image processing algorithms that enable high throughput identification of PIDs over the mouse cortex. Automated and robust algorithms, such as the one developed, are essential for analyzing the large datasets involved in studies that compare PIDs between different groups of mice. Methods: Ten mice (male C57Bl6/J, 12-14 weeks old) were anesthetized with 0.75% isoflurane and imaged up to 6 hours during middle cerebral artery occlusion (MCAO). A light emitting diode operating at 478nm illuminated the skull, and diffuse reflected light was detected by a CCD camera, at a frame rate of 30 Hz (Fig 1A). The diffuse reflectance was corrected for baseline by mean-subtraction. Results: PIDs were detected by applying several criteria to create an image mask over the trial (Fig 1B). In addition to detecting PIDs, the algorithm also calculated the duration, average velocity, and spatial trace of the PID. After the automated identification of PIDs, the user can inspect the intensity trace (Fig 1C), the spatial location of the mask over the trial (Fig 1D), and the trace of the PID (Fig 1D) to identify potential false positives and false negatives at around five times faster than manually watching all trials. Conclusion: Our algorithm automatically detected PIDs that occurred after MCAO in mice. During the 50 hours of data analyzed, there was a total of 208 PIDs. The sensitivity and specificity of the algorithm was 0.93 and 0.97 respectively, and there was a false positive and false negative rate of 0.11 and 0.07. Our imaging system and processing algorithm efficiently identify PIDs over mouse cortex, and provide quantitative information about the propagation of PIDs that cannot be calculated by manual inspection.

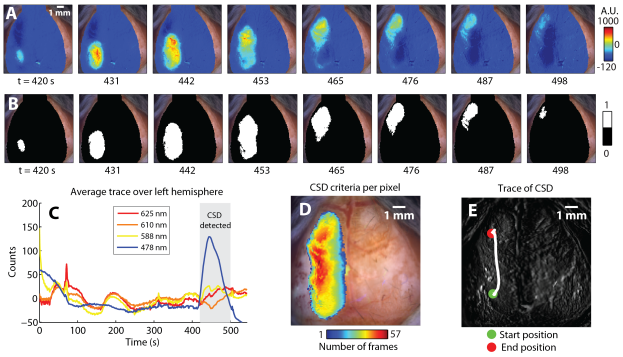

Figure 1. PID detection in mouse cortex after MCAO. **A.** Image sequence of PID in mouse after MCAO. The PID propagates at an average velocity of 3.5 mm/min from posterior to anterior. **B.** Image sequence of mask generated after passing all criteria of the detection algorithm. **C.** Average light intensity over the left hemisphere over time. Time traces of four different color LEDs are shown. In gray is the time epoch in which the algorithm detected the PID shown in A & B. **D.** The number of frames that each pixel satisfied the PID detection criteria. **E.** Trace of the PID over the mouse cortex. The start position is in green and the end position is in red.

Disclosures: J. Bumstead: None. **A. Kraft:** None. **X. Yang:** None. **A. Bauer:** None. **J. Culver:** None. **J. Lee:** None. **J. Lu:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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OGS

TWRI Fellowship

Title: The spatial and temporal distribution of immature, proliferating and mature oligodendrocytes in a rat intracerebral hemorrhage model

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Abstract: Following intracerebral hemorrhage (ICH), many brain cells die and many surviving neurons are damaged. Several aspects of the damage to grey and white matter have been characterized in animal models of ICH, and it appears that neurons in the peri-hematoma region can survive. However, little is known about re-myelination, which will be important for potential functional recovery after ICH. Re-myelination will require healthy, fully differentiated

oligodendrocytes, which are the myelin-producing cells. Our lab has previously investigated white-matter damage and inflammation during the first week after experimental ICH, but it is not known how ICH affects oligodendrocytes. Here, we analyzed the spatial and temporal changes in oligodendrocyte numbers and differentiation state at 1, 3, 7, 14 and 28 days after inducing ICH by stereotaxically injecting type IV collagenase into the rat striatum. The total number of oligodendrocytes (Olig2+ cells, identified using immunohistochemistry) in the peri-hematoma region increased after ICH and peaked at 7 days, at which time they formed a distinct band around the lesion. Closer examination of this band of oligodendrocytes showed that their numbers increased specifically inside white-matter bundles. Oligodendrocyte numbers declined by 14 days; and to determine whether this decrease was due to cell death, they were co-stained for TUNEL and cleaved caspase-3. To further characterize the oligodendrocyte population, we analyzed numbers of oligodendrocyte precursor cells (OPCs, identified as NG2+Olig2+ cells) and mature oligodendrocytes (identified as CC-1+Olig2+ cells). Numbers of OPCs and mature oligodendrocytes both peaked at 7 days. We then compared the spatial distribution of OPCs and mature oligodendrocytes inside white-matter bundles with those in the surrounding neuropil of the peri-hematoma. Interestingly, oligodendrocyte proliferation (identified as Olig2+Ki67+ cells) was elevated in the peri-hematoma at 3 days post-ICH. In contrast, there was no change in oligodendrocyte proliferation in the subventricular zone, a major site of normal oligodendrogenesis. Taken together, our findings suggest that local proliferation near the hematoma helps replenish the oligodendrocyte population after ICH.

Disclosures: **M.J. Joseph:** None. **J. Caliaperumal:** None. **L.C. Schlichter:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Title: Caffeine and/or hypothermia intervention for neonatal hypoxic-ischemic injury: effects on behavioral outcomes in a rat model

Authors: ***H. M. CONTRERAS**[1], A. L. SMITH[1], A. R. RENDALL[1], T. S. ROSENKRANTZ[2], R. H. FITCH[1];
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Abstract: One of the most common injuries among term and preterm/very low birth weight (VLBW) infants is hypoxia-ischemia (HI; reduced blood oxygenation and/or flow to the brain). Following an HI insult, an array of deficits are often seen, including motor impairments (e.g., cerebral palsy), and cognitive deficits including learning/memory, attention, sensory processing and language impairments. Caffeine and hypothermia have each been individually proposed as possible treatments for neonatal HI, and hypothermia is routinely used to treat term infants at risk for hypoxic-ischemic encephalopathy. Although magnitude of benefit varies depending on severity of insult, method (head vs whole body hypothermia), core temperature, post-injury delay, and other variables, there is substantial evidence showing improvements in overall outcome. However, cooling has not been trialed in preterm infants, due to concerns regarding co-morbid health issues. In preterm/VLBW infants, caffeine therapy has been effective for treating apnea of prematurity, and has secondarily been found to improve rate of survival. Potential benefits from the use of caffeine and hypothermia together in preterm infants remain largely unknown. Previous studies in our laboratory using Rice-Vannucci rodent HI model have revealed significant deficits in rapid auditory processing and spatial learning in male rats with postnatal day 1 (P1), P7, or P10 HI injury. Moreover, these deficits have been found to be attenuated by caffeine treatment when administered immediately following the induction of HI. The current study sought to investigate the potential therapeutic effects of caffeine citrate and hypothermia -- both alone, and in combination -- in a preterm rodent HI model. On (P) 6, HI injury was induced (cauterization of the right common carotid artery, followed by two hours of 8% oxygen). Male sham animals received only a midline incision with no manipulation of the artery, followed by room air exposure for two hours. Subsets of HI and sham animals then received either an intraperitoneal injection of 10 mg/kg of caffeine citrate, or saline immediately following hypoxia, 24 and 48 hours after. Following the first administration of caffeine citrate or vehicle, animals were assigned to hypothermic or normothermic conditions for 3 hours. All animals later underwent a battery of behavioral test that included rotarod, rapid auditory processing, Morris Water Maze (spatial and non-spatial), novel object recognition, and five choice serial reaction time task. Putative behavioral outcome benefits from intervention with caffeine, hypothermia, and caffeine + hypothermia in a late preterm HI model will be reported.

Disclosures: H.M. Contreras: None. **A.L. Smith:** None. **A.R. Rendall:** None. **T.S. Rosenkrantz:** None. **R.H. Fitch:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 687.14/I46

Topic: C.08. Ischemia

Support: CIHR Grant

OGS

Title: Is infarct location a predictor of the degree of post-stroke motor recovery?

Authors: *S. KARTHIKEYAN[1,2], M. JEFFERS[2], A. CARTER[2,3], D. CORBETT[2,3];
[1]Neurosci., [2]Univ. of Ottawa, Ottawa, ON, Canada; [3]Canadian Partnership for Stroke Recovery, Ottawa, ON, Canada

Abstract: Stroke is a leading cause of neurological disability and a majority of patients have long-term motor impairments, often as a result of damage to the motor cortex and/or striatum. While both humans and animals show spontaneous recovery following stroke, little is known about how injury location affects the recovery process. This information is essential in order to develop new therapies to enhance recovery. In this study we used endothelin-1 (ET-1), a potent vasoconstrictor, to produce focal infarcts in the forelimb motor cortex, the dorsolateral striatum or both the cortex and striatum in male Sprague-Dawley rats. The spontaneous recovery profile of the animals was followed over an 8-week period using four behavioural tasks assessing motor function and limb preference to identify how recovery varies depending on injury location. Infarct volumes were derived from MRI 72 hours post-stroke. All three models resulted in functional deficits on the Montoya staircase ($p < 0.002$), beam ($p < 0.017$), and cylinder ($p < 0.001$) tasks but no significant impairments were seen in the adhesive removal task. The three groups demonstrated distinct patterns of recovery on the behavioural tasks with the combined cortical plus striatal group having the largest and most persistent impairments overall. There were no significant differences between groups for total hemispheric infarct volume. These results suggest that damage to the striatum is an important predictor of the level of post-stroke motor impairment. Moreover, the pattern of recovery is not simply dependent on lesion volume but on lesion location and the behavioural test employed. All three models produce sustained motor impairments that will be valuable in assessing novel, adjunctive post-stroke therapies.

Disclosures: S. Karthikeyan: None. **M. Jeffers:** None. **A. Carter:** None. **D. Corbett:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Topic: C.08. Ischemia

Support: 1R01 HD049792

Title: Evaluation of compensatory re-organization and damage to the contralateral hemisphere following experimentally induced neonatal hypoxic ischemic brain injury in rats

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Abstract: The most common cause of neurological morbidity and infant mortality in the preterm/term infant is hypoxia ischemia (HI; a lack of blood and/or oxygen to the brain). In the preterm, HI may occur through intra-cranial bleeding or deficiencies in circulatory regulation, while in the term infant HI often results from prolonged labor, placental dysfunction, or other adverse birth events. Despite different etiologies, HI in either population can lead to gray and/or white matter brain damage, and subsequent cognitive/behavioral deficits. Most notably, cortical and hippocampal damage frequently underlie subsequent impairments in speech and language, motor abilities, attention, memory, and spatial orientation. However, when damage occurs early in development, the plasticity of the neonatal brain appears to allow for re-organization and/or compensation that may minimize later deficits. In fact, brain injury during this time is typically subtle, and associated behavioral deficits can often be ameliorated. This stands in contrast to the adult brain, where localized brain injury typically leads to very robust behavioral deficits (aphasia, hemiplegia, etc.), and the brain is less likely to elicit compensatory mechanisms following injury. To assess possible compensatory mechanisms following a neonatal HI insult, the current study used morphometry to measure brain tissue from rats with experimentally induced unilateral hypoxia ischemia on postnatal day (P) 7 (roughly equating to injury in the late preterm infant). Numerous studies using the Rice-Vannucci HI model have reported cortical and hippocampal damage, predominantly on the side ipsilateral to insult. The contralateral (left) hemisphere often reveals no measurable damage, despite being exposed to hypoxia (a component of the HI injury protocol). Also, though neonatal HI subjects consistently exhibit reduced right hemisphere volumes, studies have shown that these same animals are able to perform behavioral tasks with surprising levels of competency. Based on this convergent data, we sought to assess compensatory mechanisms in the contralateral hemisphere by measuring the size of various cortical and hippocampal regions in the "unaffected" hemisphere of P7 HI injured rats. Our intent was to determine whether the contralateral hemisphere sustains subtle HI damage similar to the ipsilateral hemisphere, or alternately, displays an increase in connectivity/thickness reflecting compensation in the injured neonatal rodent brain.

Disclosures: A.L. Smith: None. **T.S. Rosenkrantz:** None. **R.H. Fitch:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Topic: C.08. Ischemia

Support: R01 HD061363

Title: Crossed cerebellar atrophy of the lateral cerebellar nucleus following endothelin-1 induced stroke

Authors: *H. H. CHAN[1,2], J. COOPERRIDER[2], A. LIU[2], H. PARK[2], J. T. GALE[2], A. G. MACHADO[2];
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Abstract: Crossed cerebellar diaschisis (CCD) is a functional deficit of the cerebellar hemisphere contralateral to a lesioned cerebral hemisphere. This deficit results from the reduction of afferent input from the injured cerebral region through the corticopontocerebellar pathway. Clinically, CCD manifests as a reduction of blood flow and metabolism at the contralateral cerebellar hemisphere. Furthermore, there are also anatomical defects detected in the cerebella, termed as crossed cerebellar atrophy (CCA). In this study, we demonstrate that there was CCA, specifically in the lateral cerebellar nucleus (LCN), following an ischemic stroke in rats. Six intracortical injections of endothelin 1 (ET-1) were applied to the motor cortex of eight male Long Evans rats for induction of ischemic stroke, while another eight served as uninjected controls. Experimental rats were sacrificed six weeks after stroke induction. Cerebral sections with the motor cortex and cerebellar sections with the LCN were prepared for analyses, including Nissl staining, TUNEL assay and immunohistochemistry. Focally injected ET-1 induced a significant stroke lesion at the motor cortex, accompanied by a reduction of volume of the contralesional LCN. Apoptosis (TUNEL) was observed at the perilesional motor cortex and LCN, both ipsilesionally and contralesionally. Immunohistochemically, the densities of glutamatergic (VGluT-1) and GABAergic (GAD) neurons of the contralesional LCN were lower than those of the ipsilesional LCN. Moreover, there was a higher density of astrocytes (GFAP) at the ipsilesional motor cortex and contralesional LCN than the opposite sides. These findings suggest that cortical ischemia induced bilateral atrophy of the LCN. The higher severity of atrophy of the contralesional LCN compared to the ipsilesional LCN confirmed that the CCA of the LCN was induced by the unilateral stroke lesion at the motor cortex. This atrophy was associated with astrogliosis, implying an inflammatory mechanism.

Disclosures: **H.H. Chan:** None. **J. Cooperrider:** None. **A. Liu:** None. **H. Park:** None. **J.T. Gale:** None. **A.G. Machado:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

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Topic: C.08. Ischemia

Support: IT 773/13 Eusko Jaurlaritza

BFI-2011-129 Eusko Jaurlaritza

Title: Docosahexanoic acid improves mitochondrial funcionality and integrity in neonatal brain after hipoxia-ischemia

Authors: *E. HILARIORODRIGUEZ, M. REVUELTA, L. URIGÜEN, A. ALVAREZ, O. ARTEAGA;
Univ. of the Basque Country, Leioa, Spain

Abstract: One of the most common causes of mortality and morbidity in children is perinatal hypoxia-ischemia, so new and more effective neuroprotective strategies are urgently required, in order to minimize as much as possible the neurological consequences of this encephalopathy. In this sense, interest has grown in the neuroprotective possibilities of docosahexanoic acid (DHA), which is a long-chain omega-3 fatty acid, commonly found in fish such as tuna and salmon. The aim of the present work was to evaluate the protective effects of the docosahexanoic acid when administered before HI brain injury in neonatal rats using the Rice-Vannucci model. Hypoxic-ischemic brain injury was provoked by permanent ligature of the left common carotid artery and then by asphyxia for 135 minutes with 8% O2 when rats was P7. Then they were randomly assigned to: control, hypoxia-ischemia (HI) and HI animals that received a single dose of 1 mg/kg of DHA 10 minutes before hypoxia (HI+DHA). P14 brains were stained with Nissl and immunolabelled with MBP and mitochondrial state were evaluated by flow cytometry. Mitochondrial integrity was determined by using the fluorocrome Nonyl Acridine Orange (NAO) and mitochondrial transmembrane potential was analyzed by Rhodamine 123 (Rh 123). HI animals showed swollen and deformed neurons especially in the ipsilateral CA 1 and CTX areas, while only mild cell loss and a few damaged neurons were observed in slices from animals pretreated with DHA. A substantial loss of ipsilateral MBP immunostaining was observed in external capsule and striatum areas of animals that underwent hypoxia-ischemia respect to controls, but DHA pretreatment avoided is loss. Regarding mitochondrial integrity, at 12 h, HI group underwent a diminishment with statistical differences with respect to the Control group in the percentage of NAO positive cells, while animals pretreated with DHA maintained

mitochondrial integrity. In regards to mitochondrial transmembrane potential, animals subjected to the HI event underwent an important diminishment in the percentage of Rh 123 positive cells, but rats pretreated with the antioxidant showed similar values to those of the controls. Our results suggest that docosahexanoic acid led to neuroprottective effects by ameliorating cell damage, preserving myeling and by maintaining mitochondrial integrity and functionality.
Acknowledgments: This work was supported by grant from the Basque Government IT 773/13 and BFI-2011-129.

Disclosures: E. Hilariorodriguez: None. **M. Revuelta:** None. **L. Urigüen:** None. **A. Alvarez:** None. **O. Arteaga:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 687.18/J2

Topic: C.08. Ischemia

Title: New animal model for brain edema

Authors: *N. MATSUKI[1], T. KITAGAWA[1], Y. HOSHI[1], Y. IKEDA-MATSUO[2], R. KOYAMA[1], Y. IKEGAYA[1];
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Abstract: Brain edema is characterized by an excess fluid accumulation within the brain tissue generally caused by ischemia. In spite of its high mortality of patients with edema in the cerebellum and brain stem, the mechanism is poorly understood, and no effective treatment has been established. One of the reasons is a lack of proper animal model corresponding to the early stage of the edema. Therefore, we have established an *in vitro* brain edema model and analyzed its mechanism. Acute brain slices of young adult mice were exposed to oxygen-glucose deprivation condition (OGD) for 10 min. The cross-sectional area of the slice was measured for 150 min with a macro-microscope. Water content of slices was also measured after the experiment. After OGD treatment, the cross-sectional area of slices increased to 120% at 150 min. This increase was dependent on temperature and inhibited by an NMDA receptor antagonist or hyperosmolar treatment. Water content was also increased. These facts suggest that the model adequately replicates some features of brain edema. OGD-induced edema was significantly reduced in aquaporin4-deficient mice. Hyponatremic treatment was also preventive. A Na+/K+ ATPase inhibitor alone induced edema without OGD. These results suggest that accumulation of

intracellular sodium is involved in an early stae of the formation of brain edema. The model can contribute to elucidate the nature of brain edema and to develop effective treatments.

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Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.08. Ischemia

Support: RGC GRF HKU773210M

Title: Vulnerable vasculature and inflammation contribute to the exacerbation of transient focal ischemia in a genetic mouse model of type 1 diabetes

Authors: *A. C. LO[1,2], A. K. W. LAI[1];
[1]Dept. of Ophthalmology, The Univ. of Hong Kong, Hong Kong, Hong Kong; [2]Res. Ctr. of Heart, Brain, Hormone and Healthy Aging, The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: PURPOSE: Epidemiological studies showed that type 1 diabetic patients are much more prone to cerebrovascular mortality from stroke and the median survival is only half when compared with those in the general population. It has been suggested that type 1 diabetes is a risk factor for stroke; however, the underlying mechanisms are still unclear. In the current study, we aim to elucidate the potential mechanisms contributing to the exacerbation. METHOD: $Ins2^{Akita/+}$ mice, a type 1 diabetic murine model, and their wildtype ($Ins2^{+/+}$) littermates at 12 weeks of age were challenged with experimental stroke by middle cerebral artery occlusion (MCAO) for 2h followed by 2h of reperfusion. Survival rate and neurological deficits were accessed at the end of reperfusion. Brain slices were prepared and stained with 2, 3, 5-triphenyltetrazolium chloride for estimating the infarction, hemispheric swelling, and hemorrhagic area. Blood vessel integrity (ZO-1) and inflammatory response (VEGF and pErk) were compared between $Ins2^{Akita/+}$ and $Ins2^{+/+}$ ipsilateral brains using Western blot analysis. ER-stress (ATF6, BiP, CHOP, PERK and IRE-1α) and autophagy (Atg12, Bcn1, LC3-a, LC3-b and p62) response were also compared using real-time PCR. RESULTS: $Ins2^{Akita/+}$ mice showed a decreased survival rate and increased neurological deficits after MCAO, together with a significant increase in infarction and

hemorrhage size. Down-regulation of ZO-1 protein and remarkable up-regulation of VEGF and pErk protein were observed in $Ins2^{Akita/+}$ mice when compared with $Ins2^{+/+}$ mice. mRNA expression of CHOP was significantly increased in both mice after MCAO challenge and was further augmented in $Ins2^{Aktia/+}$ mice. Atg12, Bcn1 and LC3-b mRNA expressions were significantly lower in the $Ins2^{+/+}$ mice after MCAO when compared with the sham-operated controls but there was no difference between the post-MCAO $Ins2^{Akita/+}$ and $Ins2^{+/+}$ groups. CONCLUSION: We showed that induction of MCAO in $Ins2^{Akita/+}$ mice could mimic the clinical observations of high mortality in type 1 diabetic patients upon stroke. Decrease in ZO-1 expression and augmented hemorrhage indicated that blood vessel integrity was more vulnerable in the $Ins2^{Akita/+}$ mice. Provoked inflammatory response and ER-stress may play important roles in the exacerbation of the ischemic brain, which was evidenced in increased infarction in $Ins2^{Akita/+}$ mice.

Disclosures: **A.C. Lo:** None. **A.K.W. Lai:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Topic: C.08. Ischemia

Support: Basque Government IT 773/13

Basque Government BFI-2011-129

Title: Resveratrol improves long-term memory impairments and neuronal conexions in hypoxia-ischemic brain injury in neonatal rats

Authors: O. ARTEAGA, M. REVUELTA, L. MARTINEZ-MILLAN, L. URIGÜEN, A. ALVAREZ, A. MARTINEZ-IBARGÜEN, *J. PINEDA, E. HILARIO;
Univ. of the Basque Country-UPV/EHU, Leioa (Bizkaia), Spain

Abstract: Neonatal hypoxic-ischemic brain injuries involve primary destructive events, but also secondary maturational disturbances, which lead to subsequent abnormal development and altered functions, such as severe neurological handicap, and behavioral and learning deficits. Resveratrol (RVT) is a natural pythoalexin present in grapes and red wine with anti-oxidant and anti-inflammatory properties. The aim of the present work was to investigate if short-term protective effects of reveratrol against HI brain injury in neonatal rats were also persisted in

adulthood. To this end we performed long-term behavioral tests related with memory impairments and we evaluated axonal anterograde conexions. Hypoxic-ischemic brain injury was induced by permanent ligature of the left common carotid artery and then by asphyxia for two hours and a 15 minutes with 8% O2 when rats were 7 days old (P7), and then, they were randomly assigned to three experimental groups: control, hypoxia-ischemia (HI) and HI animals that received a single dose of 20 mg/kg of resveratrol 10 minutes before the hypoxic event (HI+RVT). On P90 we evaluated the long-lasting behavioral alterations related with memory, through T-maze and novel object recognition tests. For anterograde tracing experiments, when P100 animals, biotinylated dextran amines (BDA) injections were made in left lateral of the cortex, under anesthesia. Animals pretreated with resveratrol made a similar number of correct choices in the T-maze at 40 second delay to the control ones, while HI animals made significantly fewer correct choices. In a novel object recognition test, HI rats displayed a decrease in discrimination index when compared to control animals that was fully reversed by acute resveratrol administration. Changes in the axonal connections of corticofugal neurons following neonatal hypoxic-ischemic injury were detected in HI rats in the talamun and corpus callosum, but these changes were reverted with RVT, showing similar patterns to control animals. Our results suggest that the pretreatment with resveratrol was able to improve the long-lasting cognitive deficits related with memory and the changes in the axonal connections of corticofugal neurons in the talamun and corpus callosum induced by hypoxia-ischemia.

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Poster

687. Ischemia and Hemorrhage: Animal Models

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Topic: C.08. Ischemia

Support: NRF-2011-0010563

Title: Effects of forced exercise on changes in expression of milk fat globule-EGF factor 8 and brain-derived neurotrophic factor in cortical infarct area after focal cerebral ischemia in rats

Authors: Y.-W. CHOI[1,2], S.-G. KANG[3], *K.-Y. KAM[4,2];
[1]Rehabil. Sci., [2]U-Healthcare & Anti-aging Res. Ctr., [3]Biol. Sci., Inje Univ., Gimhae, Gyeongnam, Korea, Republic of; [4]Dept. of Occup. Therapy, Gimhae, Korea, Republic of

Abstract: After cerebral ischemia, the two major zones of injury appear in the lesion: the core and the penumbra. In the core, reduction of blood flow induces a catastrophic event due to rapid depletion of energy. However, in the penumbra, neural cells are still alive but subjected to a wave of deleterious events propagated from the core, leading to delayed cell death. According to recent studies, delayed death of stressed-but-viable neurons is executed by phagocytic microglia in ischemic region after focal cerebral ischemia. Milk fat globule-EGF factor 8 (MFG-E8) plays a role as opsonin in the phagocytosis. The neuroprotective effects of exercise are well known in recovery of damaged tissue and functions after cerebral ischemia through the mediation of brain-derived neurotrophic factor (BDNF), but its effect on MFG-E8 is still not understood. In this study, we examined the effect of forced exercise on MFG-E8 and BDNF-positive cells in the injured area after cerebral ischemia. Transient focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) on male Sprague-Dawley rats weighing 280-300g. Three days after MCAO, animals were subjected to forced treadmill exercise (20 m/min, 30 min, 1 session/day) for 14 consecutive days. The modified neurological severity score was used to evaluate neurological function at day 1, 3, 10, 17 after MCAO. The infarct size was determined by the Nissl staining. The immunohistochemical analysis was performed with antibodies against NeuN (neuronal marker), MFG-E8, and BDNF. Compared to sedentary group, exercise group showed significant improvement of neurological function and smaller infarct size. Based on the distribution of $NeuN^+$ cells, virtual borderline could be drawn to distinguish infarct core. In the peri-infarct of exercise group, the number of $MFG\text{-}E8^+\text{-}NeuN^+$ cells was significantly less than those of subacute ischemia group (3 days after MCAO) and sedentary group, while the number of $BDNF^+\text{-}NeuN^+$ cells was more than that of sedentary group. But the number of $NeuN^+$ cells in the same area was not significantly different between groups. This study suggests that the effects of forced exercise on recovery after cerebral ischemia may be mediated by alterations in actions of MFG-E8 and BDNF in the ischemic region.

Disclosures: **Y. Choi:** None. **S. Kang:** None. **K. Kam:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

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Topic: C.08. Ischemia

Support: IT773/13 Eusko Jaularitza

Title: Antioxidant treatments recover the alteration of auditory evoked potentials and reduce morphological damage in the inferior colliculus after perinatal asphyxia in rat

Authors: *A. MARTINEZIBARGUEN[1], O. ARTEAGA[2], A. ALVAREZ[2], E. HILARIO[2], M. REVUELTA[2];
[1]Dermatol. Ophtalmology and Otorhinolaryngology, Univ. of Basque Country Med. Sch., Getxo, Spain; [2]Cell Biol., UPV/EHU, Leioa, Spain

Abstract: Despite improvements in neonatology, perinatal hypoxic-ischemic (HI) encephalopathy remains one of the main causes of disabilities in term-born infants. This pathology underlies many neurological disorders such as learning difficulties, language and attention deficit, hyperactivity disorders and cerebral palsy. Moreover, it is a notable risk factor for hearing impairments which affect neonates. The aim of the present work was to evaluate morphologically and electrophysiologically the effect of a panel of antioxidants on HI-induced auditory deficits. To this end, we studied the effects of nicotine, melatonin, resveratrol and DHA on the neonatal auditory system via measurement of auditory evoked potentials and characterization of the morphological integrity of the Inferior Colliculus (IC). The hypoxic ischemic event was induced in perinatal rat, by the Rice-Vannucci method. Rats were randomly assigned to six experimental groups (n=8): control, HI and four HI groups administered with the different drugs. We measured the Auditory Brainstem Responses (ABR) and after ABR recordings, animals were weighed and sacrificed for the histological and cellular studies. Astrogliosis and white matter injury were evaluated by GFAP (glial fibrillary acidic protein) and MBP (myelin basic protein) staining and membrane integrity, potential and intracellular ROS were analyzed with a flow cytometer. We found that the integrity of the auditory pathway in the brainstem was altered as consequence of the HI insult. Thus, the auditory brainstem response showed increased I-V interval and latencies III, V waves. At a histological level, hypoxia-ischemia altered the morphology of the inferior colliculus: neurons had altered axonal prolongations and condensed cytoplasm; astrocytes were reactive with increased glial fibrillary acidic protein expression, and oligodendrocytes exhibited reduced myelin basic protein expression. Following antioxidant treatment, ABR intervals were restored and the body and brain weight was recovered as well as the morphology of the inferior colliculus that was similar to the control group. Our results support the hypothesis that antioxidant treatments have a protective effect on the functional changes of the auditory pathway and on the morphological damage which occurs after hypoxic ischemic insult. Acknowledgments: This work was supported by grants from the Basque Country Government (IT773/13) and Jesús de Gangoiti Barrera Fundaction.

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Poster

687. Ischemia and Hemorrhage: Animal Models

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Topic: C.08. Ischemia

Support: NIH Grant 5R01HD06163

Title: Modulation of the lateral cerebellar nucleus during motor learning

Authors: *J. COOPERRIDER, J. T. GALE, R. GOPALAKRISHNAN, H.-J. PARK, C. WATHEN, H. H. CHAN, A. G. MACHADO;
Cleveland Clin., Cleveland, OH

Abstract: Our group has previously investigated the local field modulation of the rat lateral cerebellar nucleus (LCN) during a skilled reaching task, identifying differences between successful and unsuccessful reaching attempts. Because there is evidence of motor learning-dependent modulation in the cerebellum, we wished to examine the activity of the LCN while animals learned this skilled reaching task. Local field potential and multi-unit spiking activity was acquired from a five-microelectrode array in the LCN of seven animals. Analysis of neurophysiological data revealed that there were significant modulatory changes during successful and unsuccessful trials over the course of learning a skilled reaching task.

Disclosures: J. Cooperrider: None. **J.T. Gale:** None. **R. Gopalakrishnan:** None. **H. Park:** None. **C. Wathen:** None. **H.H. Chan:** None. **A.G. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire, Boston Scientific, ATI, Functional Neuromodulation, Cardionomics.

Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 687.24/J8

Topic: C.08. Ischemia

Title: Intrauterine ischemia and systemic inflammation: a model of encephalopathy of prematurity

Authors: *B. CARUSILLO, V. GERZANICH, J. SIMARD;
Univ. of Maryland-Baltimore, Baltimore, MD

Abstract: Complications preceding premature birth are complex and multifactorial. These complications, including maternal infection and hypoxia-ischemia, can lead to several neurological pathologies in newborns such as Periventricular Leukomalacia and Intraventricular hemorrhage and other non-hemorrhagic brain lesions, collectively referred to as "Encephalopathy of Prematurity." Currently, this multifaceted perinatal injury is not adequately replicated in animal models. Here, we propose a novel model of encephalopathy of prematurity in which insults are induced *in utero*. Intrauterine ischemia (IUI) was induced at E19 in pregnant dams. Following IUI, an osmotic pump was implanted to deliver 600ng/hr Lipopolysaccharide (LPS) for 24 hours into the maternal circulation to mimic systemic bacterial infection. After 2-3 days, birth occurred naturally. Pups were studied from P1-P50 for histological and behavioral phenotypes. At Post-natal day 1 (P1), H&E stains showed cortical and intraventricular hemorrhages in injured pups. Additionally, these pups showed marked microglial activation in periventricular areas as evidenced by increased ED1 and IBA1 immunohistochemical labeling in those areas. Behaviorally, injured pups showed motor deficits including delayed righting reflex and negative geotropism from P1-P14 and worse performance on beam balance, rearing, and grip test at P24-P31. In the Morris Water Maze, injured pups spent significantly less time in the correct quadrant during the memory probe. Additionally, these pups displayed heightened anxiety in the Elevated Plus Maze and Open Field tests. The present rat model of transient *in utero* ischemia and maternal systemic inflammation accurately reflects the clinical events that often precede prematurity and the development of later neurological deficits. Pups from injured mothers showed marked inflammation and intracerebral hemorrhage in early post-natal life and displayed persistent motor and cognitive deficits throughout development.

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Poster

687. Ischemia and Hemorrhage: Animal Models

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Support: MOST-102-2314-B-038 -025 -MY3

Title: Neuroprotective effects of (-)-Phenserine against ischemia/reperfusion injury through the ERK-1/2 signaling pathway *in vitro* and *in vivo*

Authors: *J.-H. LAI, ESQ;
Taipei Med. Univ., Taipei, Taiwan

Abstract: **Background:** It is well known that stroke mainly leads to adult disability and death in global. The acetylcholinesteras (AChE) are upregulated and connected with inflammation and apoptosis after stroke. Recent studies have suggested that the inhibition of AChE ameliorates the ischemia-reperfusion injury and has neuroprotection. (-)-Phenserine, a selective AChE inhibitor has be demonstrated the nuroprotective properties. However, the protective effects and detail mechanisms of (-)-Phenserine on rat middle cerebral artery occlusion (MCAO) model is still unclear. This study focusses on the therapeutic effects of (-)-Phenserine for stroke in rodent focal cerebral ischemia model and oxygen-glucose deprivation/reperfusion (OGD/RP) damage on SH-SY5Y cells. **Methods:** SH-SY5Y cells which were cultured under OGD/RP and treated with (-)-Phenserine, were analyzed by MTT assay, Western blotting. Moreover, to investigated the therapeutic effects of (-)-Phenserine in rat used the MCAO model and was determined by TTC staining, TUNEL staining, immunohistochemical staining and Western blotting. **Results:** The viability and MMP-9 expression in SH-SY5Y cells was reduced by (-)-Phenserine under the OGD/RP condition. (-)-Phenserine increased the BDNF and Bcl-2 expression, but decreased activated-caspase 3 level, APP and GFAP expression through ERK-1/2 signaling pathway *in vitro* and *in vivo*. (-)-Phenserine also attenuated the infraction volume, apoptosis and improved the body asymmetry. **Conclusions:** These results were shown that (-)-Phenserine could enhance the Bcl-2 and BDNF expression for inhibiting neuronal apoptosis, GFAP, and MMP-9 expression via the ERK-1/2 signaling pathway. Furthermore, (-)-Phenserine could mitigate the injury of nerve cells to against ischemia/reperfusion injury in rat models of middle cerebral artery (MCA) ligation. Key words: Ischemia/reperfusion, MCAO, (-)-Phenserine, AChE

Disclosures: **J. Lai:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 687.26/J10

Topic: C.08. Ischemia

Title: ERV, an analgesic drug, suppresses the development of brain edema after focal ischemia: Analysis using 7-T MRI

Authors: *Y. NAKAJO[1,2], Q. ZHAO[1,3], K. YAMATO[1], J. ENMI[4], H. IIDA[4], H. KATAOKA[3], J. C. TAKAHASHI[3], H. YANAMOTO[1,5];

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Abstract: "An extract derived from the inflamed cutaneous tissue of rabbits inoculated with vaccinia virus (ERV)" is a mixture of multiple, non-protein, inflammation-related biological molecules and is used an anti-allergic and analgesic drug in humans. Since ERV is known to inhibit the kallikrein-kinin system, which increases vascular permeability and causes vasogenic edema (breakdown of the blood-brain barrier [BBB]), we investigated the effect of prophylactic ERV treatment on the development of cerebral edema after focal ischemia. C57BL/6J mice were treated with vehicle (water) (n = 6) or ERV (0.27 U $\bullet kg^{-1} \bullet day^{-1}$ [the clinical dose for chronic pain in humans]; n = 6) for 21 consecutive days. At the end of the treatment, temporary focal ischemia was induced in the cortex using the three vessel occlusion (3VO) technique. Using 7-T MRI, T2-weighted image and apparent diffusion coefficient (ADC) values were measured at 5 and 24 h after reperfusion. On the basis of the findings of 2,3,5-triphenyltetrazolium chloride (TTC) staining performed at 24 h after reperfusion, regions of interest (ROIs) were set at the ischemic core, medial border area (penumbra), lateral border area (surviving area), and an area with no ischemic injury. T2 signal amplitudes at the ischemic core and penumbra in the ERV-treated mice were significantly reduced at 5 h and 24 h of reperfusion ($P < 0.01$), whereas ADC values at the ischemic core and penumbra were significantly increased only at 5 h ($P < 0.01$), compared to the vehicle-treated mice, respectively. Further, 24 h after induction of reperfusion, the infarcted lesion volume was significantly reduced in the ERV group than in the vehicle group ($P < 0.05$). Increased T2 signal amplitudes and reduced ADC values indicate vasogenic and cytotoxic edema, respectively. Prophylactic treatment with ERV significantly reduced vasogenic as well as cytotoxic edema at the ischemic core and penumbra in the ultra-acute phase (5 h) after focal ischemia and reperfusion. At 24 h, vasogenic edema was still significantly reduced at the ischemic core and the penumbra, in the ERV group.

Disclosures: **Y. Nakajo:** None. **Q. Zhao:** None. **K. Yamato:** None. **J. Enmi:** None. **H. Iida:** None. **H. Kataoka:** None. **J.C. Takahashi:** None. **H. Yanamoto:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 687.27/J11

Topic: C.08. Ischemia

Support: Heart and Stroke grant 72043506

Title: Defining an alternative murine model of cerebral ischemia: focal vasoconstriction via endothelin-1

Authors: *C. DOJO SOEANDY, F. SALMASI, J. HENDERSON;
Univ. of Toronto, Toronto, ON, Canada

Abstract: Stroke represents one of the leading causes of long-term disability and death in North America. The failure of a number of recent human clinical trials aimed at enhancing stroke therapy have highlighted the importance of developing clearly defined, reproducible models of stroke. Many current animal models of stroke depend upon anatomical impedance of local arterial sources resulting in very large cortical infarct volumes compared with that typically seen in humans, and in substantial injury to sub-cortical structures. These injuries lie in contrast to the presentation of the vast majority of human clinical strokes. Additionally, many of these models exhibit considerable variability in infarct representation due to the stochastic nature of the parent vascular bed even within genetically identical animals. In order to understand the *in vivo* mechanisms regulating programmed cell death within cortical strokes, and to effectively evaluate potential therapeutic strategies, we must strive to first develop accurate and appropriate *in vivo* models. To this end, we have examined controlled stereotactic infusion of endothelin-1 into the adult mouse cortex as a means to generate transient focal ischemia/reperfusion injury within predefined cortical regions. Use of this approach results in discreet, well-defined infarcts which are limited to the cortex. Analysis of cellular ultrastructure in these infarct regions using electron microscopy together with molecular markers demonstrate features of programmed cell death that are consistent with apoptosis. Infarct regions were indeed confined to cortical layers and consistent with diffusion-based modelling. Analysis at increasing times after injury demonstrate the progressive nature of these cortical injuries with associated functional impairment. Stereotactic endothelin-1-mediated cortical injury thus exhibits reduced variability and morbidity compared to traditional models of rodent stroke while retaining key features seen in the majority of human clinical stroke. This system is in turn currently being utilized to decipher PCD signaling interactions in control and genetically modified murine mutants.

Disclosures: C. Dojo Soeandy: None. **F. Salmasi:** None. **J. Henderson:** None.

Poster

687. Ischemia and Hemorrhage: Animal Models

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 687.28/J12

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

Support: NIH F31 NS086441

NIH R01 NS046400

Title: Specific and local overexpression of haptoglobin improves anatomical and functional outcomes in the autologous blood intracerebral hemorrhage model

Authors: *T. ESFANDIARY[1,2], J. L. LECLERC[1,2,3], A. DANG[1,2], J. SANTIAGO-MORENO[1,2], S. DORE[1,3,2,4];
[1]Anesthesiol., [2]Ctr. for Translational Res. in Neurodegenerative Dis., [3]Neurosci., [4]Neurology, Psychiatry, Psychology, and Pharmaceutics, Univ. of Florida, Gainesville, FL

Abstract: Intracerebral hemorrhage (ICH) is a stroke subtype associated with high morbidity and mortality. With breakdown of the blood-brain barrier and entry of toxic blood components and metabolites within the brain, a highly oxidative environment ensues and leads to a toxic neuroinflammatory cascade. A major cause of the debilitation following brain hemorrhage is due to the direct toxicity of blood components, namely hemoglobin (Hb), the most upstream precipitating factor in the cascade. The acute phase plasma protein haptoglobin (Hp) binds Hb and inhibits its cytotoxic, pro-oxidative, and pro-inflammatory properties. In this study, we investigated whether the local and specific overexpression of Hp would aid in the safe detoxification and clearance of free Hb, thereby protecting the neuropil from Hb-mediated oxidative stress and improving ICH outcomes. Hp was overexpressed locally within the brain using uniquely designed adeno-associated viral vectors and ICH was induced using the intrastriatal autologous whole blood injection model. Functional outcomes were assessed daily by a 24-point neurological deficit score. At 72h post-hemorrhage, mice were sacrificed and brains collected for histological staining. Hp-overexpressing mice demonstrated smaller lesion volumes ($p<0.05$) with less blood accumulation ($p<0.05$) and improved neurologic status at 24h, 48h, and 72h post-hemorrhage ($p<0.05$) when compared to an identically treated control group (n=7-11/group). Histological staining for Iba1, GFAP, heme oxygenase-1, 4-hydroxynonenal, ferric iron, and myeloperoxidase was performed and revealed: i) significantly less heme oxygenase-1 expression and lipid peroxidation, ii) a trend towards reduced peripheral neutrophil infiltration, iii) significantly increased cortical microgliosis and cortical and striatal astrogliosis, and iv) no changes in ferric iron content or striatal microgliosis. In conclusion, Hp overexpression in the brain reduces ICH-induced brain injury and improves functional outcomes. Locally modulating brain Hp levels could represent an important clinically relevant strategy for the treatment of ICH.

Disclosures: **T. Esfandiary:** None. **J.L. Leclerc:** None. **A. Dang:** None. **J. Santiago-Moreno:** None. **S. Dore:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 688.01/J13

Topic: C.10. Trauma

Support: VA Merit Review

Title: Blocking gsk-3beta increase post tbi prevents memory impairment

Authors: *S. A. FARR[1], M. L. NIEHOFF[2], V. B. KUMAR[1], J. E. MORLEY[3];
[1]St Louis Univ/VA Med. Ctr., Saint Louis, MO; [2]Div. of Geriatrics, [3]Div. of Geriatrics/Div of Endocrinol., St. Louis Univ., St. Louis, MO

Abstract: Traumatic brain injury (TBI) is a serious problem. Veterans and individuals involved in accidents often have injuries that result in learning and memory disorders. There is a great need for treatment of TBI. Here we used an animal model of TBI which produces no visible outward signs of injury, but results in learning and memory deficits. One of the chemical reactions that occur in the brain after TBI is an increase of glycogen synthase kinase-3β (GSK-3β). Elevation GSK-3β leads to the phosphorylation of Tau and increase beta amyloid production. Both of these factors are involved in memory disorders. We have developed an antisense which blocks the activity of GSK-3β. In our first study, we developed a model of closed head concussive TBI which produces no visible outward signs of injury, but results in learning and memory deficits. Mice are subjected to 30 gram weight drop from a height of 80cm. Post-injury mice are subjected to an acute neurological evaluation to verify that the TBI did not produce any neurological damage. One week post-injury mice were tested in object recognition with 24 hour delay. At 4 weeks post injury mice were tested in T-maze foot shock avoidance memory test and another object recognition test with 24 hour delay. The objects in the second object recognition test were different shapes and made of different materials from the first to ensure there was no carry over from the first test. In the first study, we established that our mice that received the TBI were impaired on learning and memory. Memory was impaired in the TBI mice at both 1 and 4 weeks post TBI in object recognition. T-maze memory was impaired at 4 weeks post-injury. In the second study, we examined the effect of GSK-3β administration 15 minutes post injury on memory at 1 and 4 weeks. Mice which received GSK-3β antisense show improved memory in both object recognition and T-maze compared to random antisense treated mice that were subjected to TBI. The current finding suggest that inhibiting the GSK-3β spike with an antisense directed at GSK-3β after TBI prevents the learning and memory impairments associated with TBI.

Disclosures: **S.A. Farr:** None. **M.L. Niehoff:** None. **V.B. Kumar:** None. **J.E. Morley:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

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Program#/Poster#: 688.02/J14

Topic: C.10. Trauma

Support: ADHS14-00003606

NIH R03 NS-077098

NIH R01 NS-065052

Science Foundation Arizona

PCH Mission Support

Title: Re-expression of developmental thrombospondins after experimental diffuse traumatic brain injury coincides with injury-induced temporal profile of synaptic markers

Authors: ***S. OGLE**[1,3,4], H. MAY[1,3], R. K. ROWE[1,3,5], B. RUMNEY[1,3,6], S. JOHNSON[2,4], P. ADELSON[1,3], J. LIFSCHITZ[1,3,5], T. CURRIER THOMAS[1,3,5];
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Abstract: Annually, 20-50% of patients with a traumatic brain injury (TBI) develop persistent neurological deficits (e.g. sensory sensitivity) over months to years post-injury. No prophylactic treatments are available to negate the onset of these deficits. In rodents, late-onset sensory sensitivity following TBI is expressed as reduced tolerance to whisker stimulation with evidence of circuit reorganization in the somatosensory thalamocortical tracts. For circuit reorganization to result in neurological deficits, synaptogenesis is necessary. During development, thrombospondin-1 and thrombospondin-2 (TSPs), predominantly astrocyte-secreted proteins, mediate synaptogenesis through the α2δ-1 subunit of the voltage-dependent calcium channel receptor (α2δ-1). However, after neurological insult in the adult CNS, re-expression of TSPs have been implicated in mediating synaptogenesis and is thought to be the source of circuit reorganization during recovery. We hypothesize that re-expression of TSPs coincides with a

distinct temporal profile of synaptogenic molecules in the thalamus that supports circuit reorganization. For this study, adult male Sprague-Dawley rats underwent sham or moderate midline fluid percussion brain injury. At eight/ten time points over 2-months post-injury, gene and protein expression of TSPs and synaptic markers were quantified from thalamic biopsies using qPCR and automated capillary westerns, respectively. Gene expression demonstrated significant changes over time for TSP-1, TSP-2, α2δ-1, and post-synaptic density-95 (PSD-95; $p<0.05$). Protein expression demonstrated a significant increase in TSP-1 that coincided with changes in the temporal profile of synaptophysin and PSD-95 protein. Analysis of protein expression of TSP-2, α2δ-1, GAP-43, and PICK-1 are ongoing. These experiments provide a temporal profile of TSPs and synaptic markers after TBI in the thalamus. Future studies aim to mitigate injury-induced synaptogenesis as an approach to alleviate chronic neurological deficits. Success of this treatment would support a paradigm shift towards prevention of late-onset morbidity, rather than treatment of the symptoms, thereby improving quality of life for countless survivors of TBI.

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Poster

688. Traumatic Brain Injury: Animal Models II

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Topic: C.10. Trauma

Support: UBACYT grant 20020120100149

PIP-CONICET grant 00323

Title: Noise-induced maladaptive anxiety-like and risk assessment behaviors were reverted by rearing periadolescent rats in an environmental enrichment

Authors: S. J. MOLINA[1], M. MICELI[1], F. CAPANI[2], *L. R. GUELMAN[1];
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Abstract: It is known that noise exposure can induce hearing loss. However, few data are available regarding its effects on extra-auditory structures such as developing Central Nervous

System. Previous studies of our laboratory showed that exposure of immature rats to moderate noise can induce anxiety-like behaviour alterations. Interestingly, rearing these animals in an enriched environment (EE) has shown to be an effective protective tool which can prevent some noise-induced behavioral changes. Risk assessment (RA) can be defined as the acts and postures serving as defensive behaviors against threatening dangers, which rodents use to cope with potentially unsafe situations, closely related to fear and anxiety behaviors. Nevertheless, no data on noise-induced RA effects have been obtained yet. Therefore, the aim of the present work was to test the effects of different schedules of noise exposure in 7-days-old rats on anxiety-like and RA behaviours as well as the potential preventive effect of EE rearing. Rats of 7 days were exposed during 2 hours to white noise (95-97 dBA), for one day (acute noise exposure, ANE) or five consecutive days (sub-acute noise exposure, SANE), using an “ad-hoc” sound camera. After weaning, groups of 3-4 rats were transferred to an enriched cage, consisting of toys, a wheel, tunnels and ramps, while other groups were placed in standard cages. One week later, rats were subjected to elevated plus maze (EPM) and open field (OF) tasks. Results showed an increase in both anxiety-like and RA behaviors in ANE rats. In contrast, a decrease in most anxiety-like behaviors was found in SANE animals, whereas an increase in RA behaviors was observed. EE rearing was fully effective in reverting RA changes in both schedules. Interestingly, while a partial reversion was observed in anxiety-like behaviors in ANE rats, the decreased anxiety-like levels observed in SANE rats were normalized when animals were reared in EE. These findings suggest that rats exposed at an early developmental age to different noise exposure schedules might be differentially affected in emotion-related behavioral performances that EE rearing was able to reverse. It seems that the rearing of young rats in an EE tend to normalize anxiety-like behaviors, in particular in SANE animals. Conversely, the maladaptive RA behavior observed in noise-exposed animals was reestablished when animals were reared in an EE. Therefore, it could be concluded that visual, social and physical stimulation during the peri-adolescence period could interact with behavioral abnormalities induced by an earlier exposure to a physical agent such as noise, generating normalized emotional and behavioral parameters.

Disclosures: **S.J. Molina:** None. **M. Miceli:** None. **F. Capani:** None. **L.R. Guelman:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

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Topic: C.10. Trauma

Support: VA Grant 1IO1RX001774

DOD W81XWH-13-2-0095

Title: Chronic impairment of cerebral blood flow in a mouse model of repetitive mild traumatic brain injury

Authors: ***C. E. LYNCH**[1,2,3], B. MOUZON[1,3,2], C. BACHMEIER[1,2,3], F. CRAWFORD[1,2,3]; [1]Neurosci., The Roskamp Inst., Sarasota, FL; [2]The Open Univ., Milton Keynes, United Kingdom; [3]James A. Haley Veteran's, Tampa, FL

Abstract: Repeated exposure to mild traumatic brain injury (mTBI), as seen in contact sports injuries, is known to predispose individuals to development of neurodegenerative diseases such as Alzheimers Disease and Chronic Traumatic Encephalopathy (CTE). CTE is characterized by deposition and hyper-phosphorylation of the microtubule-associated protein tau throughout the brain. In addition to aberrant proteinopathy, neurodegenerative diseases are often associated with cerebrovascular abnormalities, including changes in cerebral blood flow (CBF), and loss of Blood Brain Barrier (BBB) integrity. Owing to the prevalence of mTBI, there is an urgent requirement for animal models recapitulating the pathological hallmarks, cognitive deficits, and cerebrovascular components of neurodegeneration following repetitive mild head trauma. We used heterozygous transgenic hTau mice expressing all 6 isoforms of human tau on a null murine tau background, allowing for a clinically relevant investigation of the effects of repetitive mTBI (r-mTBI) on cerebrovascular mechanics in the presence of human tau. The closed-head mTBI was administered to mice under isofluorane anesthetic using a 5mm blunt metal impactor tip at a velocity of 5m/s and a strike depth of 1mm, positioned midway to the sagittal suture. We administered 2 hits every week for 3 months to replicate the incidence of mTBI that can occur over the course of a career in contact sports. We measured CBF in both hTau and wild-type mice 3 months and 7 months post-injury, respectively, using laser Doppler imaging. We observed a significant decrease in CBF in wild-type mice (10.66% ± 1.44% compared to sham) and hTau mice (8.92% ± 1.39% compared to sham) This effect of r-mTBI on CBF may provide rationale for the link between head trauma and the development of neurodegenerative disorders like CTE. We will continue to evaluate the impact of r-mTBI on the cerebrovasculature by assessing BBB integrity and examining specific vascular markers at various time-points post-injury in upcoming studies

Disclosures: **C.E. Lynch:** None. **B. Mouzon:** None. **C. Bachmeier:** None. **F. Crawford:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.10. Trauma

Support: R01-DA16736 (MPG)

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Anesthesiology Research (FG, MPG),

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Title: Time dependent changes in the brain stem of rats exposed to blast-induced neurotrauma

Authors: *F. GHODDOUSSI[1], S. V. SAJJA[2], E. L. ETNYRE[3], P. VANDEVORD[5], M. P. GALLOWAY[4];
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Abstract: Introduction: Traumatic brain injury (TBI) induced by blast overpressure waves (bTBI) is a significant clinical problem in soldiers returning from combat. Psychiatric illnesses such as depression, anxiety, sleep disturbances, memory impairment, and PTSD have a delayed expression after the bTBI event. Reliable biomarkers are non-existent for bTBI and bTBI is under diagnosed partly due to the absence of diagnostic criteria in morphology based neuroimaging modalities (e.g. CT and MRI). Therefore, using other neuroimaging modalities such as MRS and DTI may provide insight into the development of diagnostic biomarkers for bTBIof the pathology. Brain stem (BS) is particularly vulnerable to bTBI because of the proximity of the BS to the bony protrusions of the skull, when brain bounces against the skull as a result of the blast. BS nuclei regulate arousal and autonomic functions, as well as attention and short-term memory. Trauma to this area can lead to disorientation, increased sense of frustration and anger, problems with balance and movement and sleeping difficulties. Clinically BS is one of the most commonly studied anatomic structures for prognosis after TBI. Methods: We used proton magnetic resonance spectroscopy (1H-MRS) and high-performance liquid chromatography (HPLC), both *ex vivo*, to assess the neurochemical profiles in the brain stem of male rats 3 hours, 1, 2, 3 and 7 days after exposure to a calibrated blast overpressure (117kPa). Short-term and working memory impairment as well as anxiety-like behaviors was assessed by novel object recognition and light/dark box respectively. Results: In the bTBI group, anxiety-like behavior was evident from 2 d thru 3 mo and impaired memory tasks evident at 7 d thru 3 mo post insult. MRS analysis showed that GABA, myo-Inositol (INS) and Cholines [and their ratio to creatine (CRE)] were increased significantly in the BS 1d after the blast. HPLC analysis showed significant decrease in 5HIAA/5HT ratio 3Hr after the blast. Discussion: Decreased

5HIAA/5HT 3Hr after the blast suggests attenuated 5HT input to BS nuclei as an early consequence of overpressure insult. Increase in INS, choline and GABA (or their ratio to creatine) 1d after the blast respectively may represent glial activation (including membrane turnover) and a potential compensatory response (neuronal or metabolic) to glutamate excitotoxicity. Increased GABA in this region may also be associated with the diminished 5HIAA/5HT ratio. The results highlight the utility of 1H-MRS for assessment of the neurochemical status of the brain after bTBI.

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Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: C.10. Trauma

Support: NIH Grant R37HD059288

NIH Grant R01NS069629

Title: The effects of mild TBI on spatial memory: impaired discrimination of novel spatial locations rooted in altered *in vivo* hippocampal activity

Authors: *R. PATERNO[1], H. METHENY[1], B. JOHNSON[1], C. SMITH[2,1], G. XIONG[1], A. COHEN[1,2];
[1]Children Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of Pennsylvania, Perelman Sch. of Med., Philadelphia, PA

Abstract: Traumatic brain injury (TBI) can lead to significant cognitive impairment, however the underlying neurological basis for TBI-induced cognitive dysfunction remains unknown. Many lines of research have implicated the hippocampus in the pathophysiology of traumatic brain injury. Furthermore, it has been demonstrated that the hippocampus consistently plays an important role during novelty exploration. That is, where an initially presented object is moved to a new location. However, specific impairments of spatial memory after TBI have yet to be shown. To test the association of TBI and spatial memory dysfunction, we used the Lateral Fluid Percussion Injury mouse model of TBI together with a spontaneous novelty exploration task. The task used both a transient (1 h) and protracted (1 day) retention interval between the

familiarization and test phases of the task. We found that TBI animals exhibited significantly impaired discrimination of old versus new spatial locations compared to Sham control animals. *In vivo* area CA1 local field potential recording in brain injured animals demonstrated a significant decrease in broadband activity during exploratory behavior in the home cage. To further investigate how brain injury alters spatial memory, *in vivo* electrophysiological activity in hippocampal areas CA1 and CA3 was performed. These hippocampal recordings were undertaken in TBI and Sham animals during the novelty spatial navigation task to correlate the hippocampal-associated behavior to neural oscillations. These findings indicate that TBI impairs spatial memory after a protracted compared to a transient delay interval, and further explores the neural basis underlying this cognitive dysfunction.

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Poster

688. Traumatic Brain Injury: Animal Models II

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Program#/Poster#: 688.07/J19

Topic: C.10. Trauma

Support: DOD CDMRP W81XWH-11-1-0014

Title: Characterizing the role of hif-1α as a mediator of blood-brain barrier dysfunction in blast neurotrauma

Authors: W. HUBBARD[1], M. LASHOF-SULLIVAN[2], J. ECK[1], E. LAVIK[2], *P. J. VANDEVORD[1];
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Abstract: Although hypoxia inducible factor-1α (HIF-1α) is known to contribute to TBI pathology, it has not been studied in relation to blast-induced neurotrauma (BINT). Hypoxia is thought to play a key role in injury progression after blast due to impaired pulmonary gas exchange in the lung and secondary effects on cerebral vasculature (Kirkman 2011; DeWitt 2009). HIF-1α is a mediator of blood-brain barrier (BBB) disruption which is linked to detrimental effects on brain pathology (Ogunshola, 2012). This injury pathway, specifically in the amygdala, can potentially lead to neurologic impairment, such as anxiety (Sajja, 2014). Understanding the mechanism of how HIF-1α contributes to BINT could provide evidence of

BBB dysfunction following blast. Sprague Dawley rats were exposed to peak overpressure of 28 psi in a side-thorax orientation for 2.5 ms duration. Anesthetized animals were exposed to a blast from an Advanced Blast Simulator. Sham animals underwent similar procedures with the exception of the exposure to blast. One week post-blast, animals were given a behavioral task to explore an open field box (anxiety assessment) for five minutes. After the behavioral assay, animals were euthanized, brains were collected and processing. Sections from the amygdala were obtained for immunohistochemistry using HIF-1α (hypoxia indicator) and SMI-71 (BBB integrity marker). Images were taken at 20x under fluorescent filters and analyzed using ImageJ. Statistical analyzes were performed using JMP Pro 11, $p < 0.05$ considered statistically significant. Anxiety-like behavior, which was measure by the fraction of time spent at the walls of the open field box, was significantly increased in the blast group ($p<0.02$) when compared to sham. Changes in anxiety have been previously reported following blast and could be the neurological manifestation of amygdala injury pathology. Histological results showed that HIF-1α expression was increased around the major vessels in the amygdala at seven days post-blast as compared to sham. In addition, the presence of SMI-71 was decreased in the blast group compared to the sham. These results are indicative of compromised BBB integrity. Characterizing the role of HIF-1α as a systemic responder and secondary mediator after polytrauma is important in understanding injury pathways and how they can contribute to behavioral deficits. The presence of HIF-1α in blast injury modes can direct therapeutic interventions for BINT. Elucidating the temporal response of HIF-1α after BINT and subsequent inflammatory pathways will provide more understanding of injury progression.

Disclosures: W. Hubbard: None. **M. Lashof-Sullivan:** None. **J. Eck:** None. **E. Lavik:** None. **P.J. Vandevord:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

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Program#/Poster#: 688.08/J20

Topic: C.10. Trauma

Support: Army CCCRP: H_005_2012_WRAIR

Title: Longitudinal characterization of gait disturbances following repeated mild traumatic brain injury in rats

Authors: *A. MOUNTNEY, J. FLERLAGE, C. RHO, W. YANG, F. TORTELLA, D. SHEAR; Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Concussion management represents a significant clinical challenge, particularly in soldiers and professional athletes who are at increased risk of sustaining repeated concussions. The current study is part of a larger series of studies designed to evaluate the critical time-frame during which the injured brain remains most vulnerable to a second concussion. This study assessed effects of multiple concussions occurring at different intervals on sensorimotor function. Rats were exposed to a single projectile concussive impact (sPCI) injury followed by a second PCI injury at 24h ($rPCI_{24H}$) or 7 days post ($rPCI_{7D}$). Sensorimotor abnormalities were assessed using the CatWalk gait analysis system, the neurological severity score-revised (NSS-R) and rotarod. Single PCI injury produced subtle, yet significant differences in fine sensorimotor parameters such as stance, swing speed, and step cycle with no overt changes in coordination (regularity index). All PCI-injured animals showed decreased cadence, increased stance, and decreased swing-speeds. Deficits were equally distributed between the four limbs and appeared acutely, with progressive resolution over 2-7 days post-injury. Regardless of injury interval, the majority of changes were detected at 2h post-injury. By 24h post-injury, sPCI, $rPCI_{24H}$ and $rPCI_{7D}$ showed 80%, 63%, and 100% reductions in abnormal gait parameters, respectively. Critically, all PCI-injured animals exhibited a bi-phasic profile showing delayed reemergence of gait abnormalities. Specifically, at 28d the $rPCI_{24H}$ group showed the greatest number of gait alterations (19) compared to sPCI (6) and $rPCI_{7D}$ (12). Animals subjected to repeated concussion at 24h intervals displayed significant changes in interlimb support, adopting an overly reliant tri-limb walking pattern. Additionally these $rPCI_{24H}$ rats exhibited significant deficits in motor learning capacity (rotarod task) and highest NSSR scores, indicative of chronic neurologic damage compared to all other groups. Overall, we found that repeated concussion significantly altered sensorimotor function with the greatest decrements evident with two concussions occurring within 24h. Additional studies are ongoing to evaluate effects of different intervals between hits (i.e. 48 to 72h) and to identify potential correlations between sensorimotor and molecular changes. However, these initial results provide further validation for usefulness of the PCI model as a research tool to better understand the time frame during which the injured brain is more vulnerable to repeat concussions and, ultimately, to evaluate the therapeutic efficacy of promising treatment strategies.

Disclosures: **A. Mountney:** None. **J. Flerlage:** None. **C. Rho:** None. **W. Yang:** None. **F. Tortella:** None. **D. Shear:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 688.09/J21

Topic: C.10. Trauma

Support: ESRC

Title: Language impairments in traumatic brain injury: A case series

Authors: *M. HALL, L. CLOUTMAN, A. WOOLLAMS;
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Abstract: Cognitive deficits and cortical lesions are common following traumatic brain injury (TBI). Research into language deficits resulting from TBI is scarce. As there are many aspects of language controlled by various regions and language is also underpinned by executive control, it can be expected that language impairments would occur following TBI. We aim to determine if language deficits occur in this group and if they can be attributed to common cortical lesions. Four TBI patients (P1-4; age range 29-48), recruited from community groups, completed neuropsychological tasks designed to examine general cognition and various aspects of language functioning. Reaction times (RT) were also recorded. Using automated lesion identification software, participants' T1 weighted MRI images were segmented into grey/white matter images, smoothed, and compared with control data (18 healthy controls aged 18-30) to identify abnormal voxels. Lesions were grouped, defined and lesion overlay maps generated which were then compared to behavioural data. P1 and P2 had overlapping right frontal and callosal lesions. Performance was worse in general cognition, reasoning, visuospatial abilities, semantics and orthographics. P1 had increased RT globally and P2 showed increased RT for semantics. P3 also showed overlapping abnormal voxels in the corpus callosum and a small lesion in the left cerebellar lobe. Performance was impaired on executive functioning and orthographics. P4 had no lesions. However, executive performance was impaired. The results suggest that right frontal regions and the corpus callosum may play a role in semantics and orthographic processing as well as visuospatial and executive functioning. These areas may also play a role in speed of functioning. However, given the small sample size this needs to be explored further. The absence of cortical lesions in P4, despite impaired performance on executive tasks, highlights the need for more complex imaging such as diffusion tensor imaging and anatomical connectivity mapping as white matter injury may account for impaired performance.

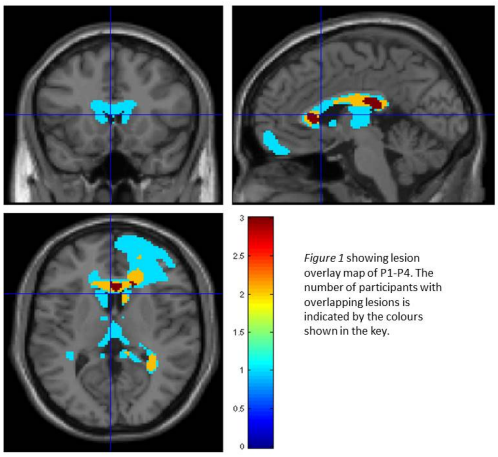

Figure 1 showing lesion overlay map of P1-P4. The number of participants with overlapping lesions is indicated by the colours shown in the key.

Disclosures: **M. Hall:** None. **L. Cloutman:** None. **A. Woollams:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

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Program#/Poster#: 688.10/J22

Topic: C.10. Trauma

Support: DoD W81XWH-12-MRPRA-CSRA

DoD W81XWH-13-CRMRP-VRP-TRA

NIH 5F31NS077796

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Crown Foundation

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NINDS U01NS086659-01

Title: Early chronic traumatic encephalopathy in young athletes after concussive head injury and a mouse model of impact concussion

Authors: ***L. E. GOLDSTEIN**[1,2,3], C. E. TAGGE[3], A. M. FISHER[3], A. GAUDREAU-BALDERRAMA[3], M. W. WOJNAROWICZ[1], O. MINAEVA[3], J. A. MONCASTER[1], N. CASEY[1], X.-L. ZHANG[4], O. MIRY[4], L. R. VOSE[4], G. SUBAH[4], K. R. GOPAUL[4], G. F.

HALL[5], R. O. CLEVELAND[6], W. C. MOSS[7], L. VELISEK[4], T. D. STEIN[8], P. K. STANTON[4], A. C. MCKEE[1,2,8];
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Abstract: The mechanisms by which head injury induces acute concussion and chronic sequelae are not known. We examined postmortem brains from young athletes after concussive head injury and found parenchymal contusion, myelinated axonopathy, microvasculopathy, neuroinflammation, neurodegeneration, and phosphorylated tauopathy consistent with early chronic traumatic encephalopathy (CTE). We developed a biofidelic mouse model of impact concussion that induces non-skull deforming head acceleration, acute concussion, and traumatic brain injury (TBI) in non-anesthetized C57BL/6 mice. Impacted mice exhibited contralateral circling, limb weakness, locomotor abnormalities, and impaired balance that recapitulates human concussion. Neurological function rapidly returned to baseline, but markers of early CTE persisted long after recovery. Impact concussion induced blood-brain barrier disruption, neuroinflammation, impaired hippocampal axonal conduction, and defective long-term potentiation (LTP) of synaptic transmission in prefrontal cortex. Kinematic analysis revealed head acceleration sufficient to induce concussion, TBI, and CTE-linked pathology. Notably, concussion did not correlate with CTE markers or chronic sequelae. Concussion was observed following impact injury but not blast exposure under conditions of comparable head kinematics. Dynamic modeling revealed greater brain shear stress during impact compared to blast neurotrauma. These results indicate that while acute concussion and chronic sequelae may be triggered by the same insult, the pathophysiological responses underpinning these effects engage distinct mechanisms and time domains. Concussion per se is neither necessary nor sufficient to trigger acute brain injury and chronic sequelae, including CTE. These results suggest that the critical variable of clinical relevance is neurotrauma exposure (hits).

Disclosures: L.E. Goldstein: None. **C.E. Tagge:** None. **A.M. Fisher:** None. **A. Gaudreau-Balderrama:** None. **M.W. Wojnarowicz:** None. **O. Minaeva:** None. **J.A. Moncaster:** None. **N. Casey:** None. **X. Zhang:** None. **O. Miry:** None. **L.R. Vose:** None. **G. Subah:** None. **K.R. Gopaul:** None. **G.F. Hall:** None. **R.O. Cleveland:** None. **W.C. Moss:** None. **L. Velisek:** None. **T.D. Stein:** None. **P.K. Stanton:** None. **A.C. McKee:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

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Program#/Poster#: 688.11/J23

Topic: C.10. Trauma

Support: DoD W81XWH-13-2-0095

VA 1IO1RX001774

Roskamp Foundation

Title: Visual dysfunction screening in mice after TBI using an optomotor assessment of the optokinetic response

Authors: *S. FERGUSON[1,2], B. MOUZON[1,2], D. APONTE[1], G. CRYNEN[1], V. MATHURA[1], M. MULLAN[1], F. CRAWFORD[1,2];
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Abstract: Introduction: Our mouse model of repetitive mild TBI (r-mTBI) produces chronic optic nerve pathology and retinal degeneration. In order to assess the visual function of the mice, we have optimized a mechanical optomotor assay to assess the optokinetic response. Methods: The optomotor apparatus consisted of a rotating drum containing black and white stripes at varying angular resolutions. Mice were acclimated to the apparatus for a period of 5 minutes in phototopic lighting. Optomotor testing at each resolution consisted of pairs of 2 minute trials with 1 trial in a clockwise rotation followed by 1 trial in a counter-clockwise rotation with an inter-trial time of 30 seconds. Following the completion of the first pair of trials in photopic conditions, lighting was dimmed to scotopic conditions and the mice were allowed to acclimate for a period of 5 minutes followed by another pair of trials. After the completion of all 4 trials the mouse was returned to the home cage and the next mouse was tested. On subsequent days this testing was repeated with the rotation of the drum increased in a range from 2 to 5 rpm. All trials were recorded with Noldus Ethovision XT. Results: Optomotor testing and optimization revealed a non-random, quantifiable optokinetic response of the mice which was found by excluding the portions of the trial where the mouse was in motion and by quantifying the angular rate of rotation of the head of each mouse. Conclusions: By varying the resolution of the stripes we were able to increase the difficulty of the task and determine the optimal conditions for eliciting an optokinetic response in healthy mice capable of discriminating subtle vision deficits. Varying the rotation rate of the optomotor drum also allowed us to determine the optimal speed for eliciting the optokinetic response. The optimized assay will allow us to accurately assess the functional outcome of potential therapeutics for the treatment of TBI-induced visual dysfunction.

Disclosures: **S. Ferguson:** None. **B. Mouzon:** None. **D. Aponte:** None. **G. Crynen:** None. **V. Mathura:** None. **M. Mullan:** None. **F. Crawford:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 688.12/J24

Topic: C.10. Trauma

Support: DOD Gran W81XWH-13-1-0384

R01ES024233

R01AG037481

R01AG037919

K01AG044490

Title: Effect of age and APOE isoform on traumatic brain injury in mice

Authors: *E. L. CASTRANIO[1], A. MOUNIER[1], J. SCHUG[2,3], N. F. FITZ[1], R. KOLDAMOVA[1], I. LEFTEROV[1];
[1]Envrn. and Occup. Hlth., Univ. of Pittsburgh, Pittsburgh, PA; [2]Functional Genomics Core, Dept. of Genet., [3]Inst. for Diabetes, Obesity and Metabolism, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability in army personnel and general population. Recent studies demonstrate that inheritance of the APOEε4 allele may lead to worse outcome following TBI. It is unknown, however, how the deficiency in ATP-binding cassette transporter A1 (ABCA1), which impacts both APOE lipidation and stability, affects outcome after TBI. This study uses mice with targeted replacement of endogenous ApoE by human cDNA for expression of APOEε3 (E3) and APOEε4 (E4), and E3 and E4 mice with only one functional copy of ABCA1 gene. The goal was to determine if age, expression of APOE isoform and ABCA1 deficiency affect cognition and gene expression following TBI. Young adult and aged mice received either a controlled cortical impact (CCI) brain injury or a craniotomy in the left hemisphere. Following three days of recovery, mice underwent multiple motor coordination and cognitive behavior tests. We found that injury causes significant differences in anxiety as tested by elevated plus maze in both young adult ($p < 0.05$) and aged ($p < 0.05$) mice regardless of APOE isoform. Injury also affected performance in Morris Water Maze ($p < 0.05$) in aged mice. In contextual fear conditioning paradigm, expression of APOE isoform had a significant effect ($p < 0.05$) in aged mice. We also found an

effect of Abca1 deficiency in some tests. At the end of the tests, mice were anesthetized and perfused, during which ipsilateral and contralateral cortical and hippocampal tissues were harvested. These tissues were used to perform gene expression analysis through RT-QPCR and RNA-Seq. The outcome of all these tests revealed differences in response to injury in both behavior and RNA gene expression dependent on genotype and age.

Disclosures: E.L. Castranio: None. **A. Mounier:** None. **J. Schug:** None. **N.F. Fitz:** None. **R. Koldamova:** None. **I. Lefterov:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 688.13/J25

Topic: C.10. Trauma

Support: UBC Centre for Brain Health Internal Seed Grant

Title: Choice impulsivity is increased following frontal traumatic brain injury in rats

Authors: *K. M. MARTENS, C. L. WELLINGTON, C. A. WINSTANLEY, C. VONDER HAAR;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Intro Traumatic brain injury (TBI) is associated with numerous consequences, including chronic changes in cognitive function. Persons with brain injury frequently show sub-optimal or even deleterious choice behavior, resulting in considerable financial, social and work-related strain. Despite the obvious problems for human patients, many animal studies have neglected the study of decision-making following TBI. Methods The current study aimed to explore shifts in impulsive choice behavior following a brain injury using the delay discounting paradigm in rats. Rats were trained on the delay discounting task for 35 sessions. Once choice behavior had stabilized, they were given either a severe (depth: 2.5 mm @ 3 m/s) or mild (depth: 0.8 mm @ 1 m/s) bilateral controlled cortical impact injury centered over the frontal cortex or given either an intact or craniotomy sham procedure to account for effects of surgery. After a week of recovery, they were re-tested on the delay discounting task. Results Both sham groups showed no change from pre-surgery choice behavior and there was no difference between craniotomy and intact shams. Severe TBI caused a large shift towards more impulsive choices, and even mild TBI produced an increase in impulsive choice, although it was less pronounced. Severe injury initially caused a high level of omitted trials which resolved to pre-injury levels by

session 5. Discussion In the current study, we show that choice impulsivity is increased in rats following a frontal TBI. This resembles reports in the clinical literature regarding impulse control problems in human patients. By examining decision-making in animals following a TBI, we are able to more closely mimic the human condition, which could lead to novel and/or improved treatments for brain injury.

Disclosures: K.M. Martens: None. **C.L. Wellington:** None. **C.A. Winstanley:** F. Consulting Fees (e.g., advisory boards); Shire - Advisory Board. **C. Vonder Haar:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 688.14/J26

Topic: C.10. Trauma

Support: CIHR Operating Grant

Title: Increased cocaine self-administration is associated with changes in neuroinflammatory markers following frontal traumatic brain injury

Authors: *C. VONDER HAAR[1], J.-M. N. FERLAND[1], L.-K. RIPARIP[2], S. ROSI[2], C. A. WINSTANLEY[1];
[1]Psychology, Univ. of British Columbia, Vancouver, BC, Canada; [2]Physical Therapy and Rehabil. Sci., Univ. of California-San Francisco, San Francisco, CA

Abstract: Intro Clinically, increased risk for drug addiction is becoming a large concern following traumatic brain injury (TBI). Recent reports have suggested that even milder injuries may lead to an increased likelihood of developing drug addiction. However, these concerns have not been evaluated in an animal model of TBI. Methods In the current study, rats were assessed on a cocaine self-administration paradigm following a mild or severe TBI. Rats received either severe (depth: 2.5 mm @ 3 m/s) or mild (depth: 0.8 mm @ 1 m/s) bilateral controlled cortical impact centered over the frontal cortex or sham procedures. After one week of recovery, rats underwent surgery to implant a catheter in the jugular vein. Following another week of recovery, they began the cocaine (or saline as control) self-administration procedure. Rats were placed in an operant chamber for six hours daily for ten days with access to cocaine infusions (0.5 mg/kg/infusion) contingent upon a lever press with a minimum of 40 seconds between infusions and a maximum of 30 infusions per hour. Following self-administration, rats were euthanized the next day and brains collected for analyses. Brains were analyzed in a multiplex ELISA to

determine the levels of various cytokines. Results Both mild and severe brain injury increased the number of presses for and total infusions of cocaine compared to sham animals. Cocaine increased the levels of all measurable cytokines in sham animals compared to saline shams. Brain injury also increased the levels of all cytokines compared to saline shams. However, cocaine in brain-injured animals actually reduced the levels of multiple cytokines relative to their saline controls. Discussion This study demonstrates a novel finding in rats that cocaine intake is increased following a brain injury and that this intake may mediate neuroinflammatory status. This provides experimental evidence for the clinical observations that risk for addiction is increased in brain injury cohorts and identifies a potential mechanism by which this may occur. Further studies will be required to investigate this outcome more fully and determine if therapeutics may be able to reduce the likelihood of addiction in patients with brain injury.

Disclosures: **C. Vonder Haar:** None. **J.N. Ferland:** None. **L. Riparip:** None. **S. Rosi:** None. **C.A. Winstanley:** F. Consulting Fees (e.g., advisory boards); Shire - Advisory Board.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 688.15/J27

Topic: C.10. Trauma

Title: Development and validation of a novel experimental animal model of post-traumatic stress disorder (PTSD)

Authors: ***N. SINGH**, D. GARABADU, S. KRISHNAMURTHY;
Indian Inst. of Technol. (BHU), Varanasi, India

Abstract: Post-traumatic stress disorder (PTSD) develops following exposure to life-threatening trauma. The high prevalence, chronicity, and resistance to treatment underscore the importance of the development of effective therapeutic strategies for PTSD. The establishment of an appropriate animal model of PTSD can elucidate the pathophysiology of PTSD which may help to identify novel and more effective therapeutic strategies. Thus, an optimal animal model would mimic the pathophysiological abnormalities and behavioral characteristics of PTSD and involve exposure to trauma-like events. No single widely accepted animal model of PTSD has been established to date. Recently, we have developed a chronic modified stress re-stress model of PTSD for the evaluation of drugs in its pharmacotherapy which exhibits most of the PTSD-like behavioral and neuroendocrinological abnormalities in rats. Further, to make the model more simpler than the earlier we have hypothesized to replace the force-swim paradigm with foot-

shock as re-stress cue in the previous experimental protocol. In the present study, two different sets of variable stressors (2 hr restraint followed by 20 min forced-swim and halothane anaesthesia (VS+FST); 2hr restraint followed by 2.0 mA shock for 10 sec and halothane anaesthesia (VS+FS)) were subjected to the animals on day-2 of the experimental animals. The re-stress paradigms for VS+FST and VS+FS were forced-swim and foot-shock respectively. Paroxetine (10.0 mg/kg, p.o.) was administered from day-8 to the day-32 of the experimental schedule. PTSD-like symptoms such as anxiety (decrease in percentage in open arm entries and time spent into open arm) during elevated plus maze test, depression (increase in immobility period) during forced-swim test and cognitive deficits (loss in spatial recognition memory) during Y-maze test induced by foot-shock as the re-stressor was higher compared to forced-swim as re-stress cue. The stress subjected rats showed lower plasma cortisone levels, a cardinal feature of PTSD. Moreover, the freezing behavior by foot-shock was elevated on day-8 of the experimental protocol and was found to be consistent throughout the experimental schedule. Paroxetine was able to ameliorate the behavioral and neuroendocrine function in the modified and novel model of PTSD. These observations emphasize the fact that foot-shock-induced PTSD-like symptoms are more precarious than forced-swim-induced PTSD-like manifestations in rats. Hence, this new animal model of PTSD could be more appropriate to evaluate the efficacy of therapeutic agents for PTSD.

Disclosures: **N. Singh:** None. **D. Garabadu:** None. **S. Krishnamurthy:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

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Topic: C.10. Trauma

Support: NJCBIR CBIR14FEL005

NJCBIR CBIR11PJT003

Title: Establishment and characterization of a rodent model of repetitive subconcussive traumatic brain injury

Authors: ***M. LONG**[1,2], A. M. FITZSIMMONS[1], K. C. H. PANG[3], V. SANTHAKUMAR[4], B. J. PFISTER[1];
[1]Dept. of Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ; [2]Rutgers Biomed. and Hlth. Sci., Rutgers Grad. Sch. of Biomed. Sci., Newark, NJ; [3]Dept. of Veteran Affairs,

Neurobehavioral Res. Lab., East Orange, NJ; [4]Dept. of Pharmacology, Physiol. and Neurosciences, Rutgers New Jersey Med. Sch., Newark, NJ

Abstract: Repetitive mild traumatic brain injury (rmTBI) and concussions are major risk factors for long-term cognitive and behavioral impairments. In animal studies rmTBI increases adverse outcomes of subsequent concussive insult. Cumulative head injuries over extended periods are implicated dementia and chronic traumatic encephalopathy (CTE). Curiously, the lack of correlation between clinical history of concussion and histological hallmarks of CTE (McKee et al., 2009) suggest that subconcussive insults may contribute to long-term damage. The paucity of information on cumulative subconcussive insults has led to little guidance in the management of repetitive scTBI within single day, a common occurrence among young adults in sports and combat. We present a model of repetitive scTBI defined by the absence of acute behavioral measures and neuronal degeneration. Wistar rats (21-25 day old) were subjected to lateral fluid percussion injury (FPI) at peak pressures 14±1.0 psi (Group 1) or 10±1.0p psi (Group 2) using a digitally controlled FPI (DcFPI) or received sham injury. Behavioral measures including apnea, righting time, toe-pinch reflex and seizures were assessed immediately after injury. Righting times pressure did not differ between groups with different injury peak pressure (one-way ANOVA, $F(2,37) = 0.550$, $p = 0.582$) Group 1: 148.4±24.65, Group 2: 122.1±25.95, n=13 each, Sham: 117.3±16.4 n=14 each. Seven of 13 group 1 rats exhibited apnea (3.75±0.46s, n=7), but none of group 2 or sham rats (n=5 each) did. Based on the presence of apnea Group 1, scTBI was modeled using FPI at peak pressure of 10 psi. To determine effects on repetitive scTBI, rats received 1, 2 or 3 subconcussive injuries at 5 min intervals. Although there was no hemorrhage or dentate hilar neuronal degeneration after single injury, rats with repetitive insults showed evidence of hemorrhage in the hippocampal fissure despite the absence of acute behavioral signs or hilar neuronal degeneration. Thus, we have a model of scTBI in young rats and demonstrated that repetitive scTBI can lead to the brain hemorrhage that could result in subsequent pathology. The repetitive scTBI model presented here is a valuable tool to assess long-term histological and behavioral deficits following multiple scTBI that occur in athletes and soldiers. Support provided by NJCBIR CBIR14FEL005 (ML); NJCBIR CBIR11PJT003 (B.J.P and V.S.)

Disclosures: M. Long: None. **A.M. Fitzsimmons:** None. **K.C.H. Pang:** None. **V. Santhakumar:** None. **B.J. Pfister:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 688.17/J29

Topic: C.10. Trauma

Support: The Roskamp Foundation

Title: A novel mouse model of chronic PTSD and repetitive mild TBI with translation to a human clinical population

Authors: *M. ALGAMAL[1,2], J. O. OJO[1,2,3], B. MOUZON[1,2,3,4], M. MULLAN[1,2], D. DIAMOND[5,3], F. CRAWFORD[1,2,3,4];
[1]Roskamp Inst., Sarasota, FL; [2]The Open Univ., Milton Keynes, United Kingdom; [3]James A. Haley Veterans' Hosp., Tampa, FL; [4]Chronic Effects of Neurotrauma Consortium, Richmond, VA; [5]Univ. of South Florida, Tampa, FL

Abstract: A novel mouse model of chronic PTSD and repetitive mild TBI with translation to a human clinical population Moustafa Algamal[1,3], Joseph O Ojo[1,2,3], Benoit Mouzon[1, 2, 3,5], Michael Mullan[1, 3] , David Diamond[2, 4], Fiona Crawford[1,2,3,5] Author Affiliation: 1 Roskamp Institute, Sarasota, Florida; 2 James A. Haley Veterans' Hospital, Tampa, Florida; 3 The Open University, Milton Keynes, United Kingdom; 4 University of South Florida (USF). 5 Chronic Effects of Neurotrauma Consortium, Richmond, VA Posttraumatic stress disorder (PTSD) is estimated to affect 20% of returning active duty soldiers engaged in recent conflicts in Iraq/Afghanistan. Very often these population are exposed to a previous history of mild traumatic brain injury (mTBI), which can double the risk for PTSD. Comorbid mTBI and PTSD can be clinically challenging to diagnose in humans, due primarily to the overlap in both conditions, and the heterogeneous nature of some clinical symptoms. This underscores the need to develop a relevant animal model that explores the effects of mTBI and PTSD. We previously developed a novel mouse model of combat related PTSD, involving exposures to predator odor, physical trauma and social instability. This model shows behavioral phenotypes at acute timepoints that are analogous to the first four criteria of the DSMV, namely: recall of traumatic memories, anxiety, and avoidance behavior. In this current study, we have modified our paradigm, including a more chronic repetitive exposure to aversive stimuli, and a new re-exposure paradigm that mimics the aspect of multiple deployments in our model. Additionally, we have included a repetitive concussive injury model into this paradigm, to explore the association between PTSD and repetitive mTBI. In this study, we perform a novel prospective analysis of our animal model on a longitudinal timescale, examining: neurobehavioral, neuroendocrine and neuroimmune phenotypes, metabolite profiles and histological outcomes, at a level that has not been previously employed, and compare these with profiles from human patients. Using multiple extended time points (2weeks, 3 and 6 months post-exposure), we address this major and often underexplored area in the field of PTSD research that has focused primarily on acute consequences of trauma. Generation of these data is currently ongoing. We anticipate that our model will be a good platform to explore targeted biomarkers and treatment strategies in PTSD and repetitive mTBI.
Key words: repetitive mild traumatic brain injury, posttraumatic stress disorder, animal models, neurobehavior, and biochemistry. This study is funded by the Roskamp Foundation.

Disclosures: **M. Algamal:** None. **J.O. Ojo:** None. **B. Mouzon:** None. **M. Mullan:** None. **D. Diamond:** None. **F. Crawford:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

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Program#/Poster#: 688.18/J30

Topic: C.10. Trauma

Support: Doctoral Program in Molecular Medicine (DPMM), University of Eastern Finland

European Science Foundation

Acadamy of Finland

Title: Transcriptomics of antiepileptogenic targets after traumatic brain injury

Authors: ***A. LIPPONEN**, M. HILTUNEN, J. PAANANEN, N. PUHAKKA, A. PITKÄNEN; Univ. of Eastern Finland, Kuopio, Finland

Abstract: Traumatic brain injury (TBI) is estimated to cause about 10-20% of acquired epilepsies. We hypothesized that transcriptional changes after experimental TBI can be used to screen potential targets for antiepileptogenic (AEG) treatments. TBI was induced with lateral fluid-percussion injury in adult rats. At 3 months post-TBI coronal slices were sectioned to sample the perilesional cortex, thalamus and hippocampus for transcriptome sequencing (RNA-Seq). In the cortex, we found differential expression in 4964, in the thalamus in 1966, and in the hippocampus in 1 gene as compared to controls (adj. $p<0.05$). Biological processes assessed with Gene Set Enrichment Analysis (GSEA) indicated down-regulation of neuronal and ion transport related gene sets and up-regulation of immunity related gene set both in the cortex and thalamus. In the hippocampus, we found a down-regulation in 1 axon guidance gene set. Expression of targets which have previously shown a disease-modifying effect on epileptogenesis in experimental models (n=52) were compared to differentially expressed genes. In the cortex 24 (16 down-regulated, 8 up-regulated) and in the thalamus 9 (5 down-regulated, 4 up-regulated) of the candidate AEG targets had altered expression after TBI. These data show a long-lasting downregulation of genes encoding ion channels and upregulation of genes involved in immune response after TBI. Moreover, majority of the candidate AEG targets were down-regulated at 3 months post-TBI.

Disclosures: **A. Lipponen:** None. **M. Hiltunen:** None. **J. Paananen:** None. **N. Puhakka:** None. **A. Pitkänen:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 688.19/J31

Topic: C.10. Trauma

Support: NIH Grant P30DA033934

Title: Traumatic brain injury elicits hemisphere-dependent motor and memory deficits in C57BL6/J mice

Authors: ***L. D. O'BRIEN**[1], T. M. REEVES[2], A. J. MORALES[1], A. H. LICHTMAN[3]; [2]Anat. and Neurobio., [3]Pharmacol. and Toxicology, [1]Virginia Commonwealth Univ., Richmond, VA

Abstract: Traumatic brain injury (TBI) represents a significant public health problem characterized by clinical cognitive impairments, such as learning and memory deficits. Left-right hippocampal asymmetry has been described in humans, which allows for functional specialization of memory. Murine left-right asymmetries have also been previously noted in hippocampal receptor morphology in mice, showing glutamate receptor content differences from afferents originating in the left or right CA3. Also, hemispheric asymmetries have been reported in long term potentiation of the CA3-to-CA1 system. Here, we examined whether lateral fluid percussion injury over the left or right parietal cortex of C57BL6/J mice produces hippocampal asymmetry of memory function. To address this question, we subjected mice to a moderate/severe (1.92-1.94 atm) injury, and measured impairments in hippocampal-dependent spatial memory tasks of the Morris water maze: 1) acquisition in the Fixed Platform task, and 2) cognitive flexibility in a Reversal task. The expression of spatial memory was assessed in both tasks using a Probe Trial, in which spatial preferences within the maze were measured without access to an escape platform. We also assessed post-injury righting time (a reliable correlate of injury severity), and neurological motor impairments using the Neurological Severity Score (NSS) and the Rotarod task. Left and right lateral injuries produced respective righting times of 391.8±54.3 and 415.0±81.3 s (mean±SEM), significantly greater in magnitude than respective sham control groups, 73.1±6.4 and 76.6±7.9 s (mean±SEM). In the Morris water maze, both injury groups showed similar acquisition rates in the Fixed Platform task as compared to sham controls, but displayed impaired search strategies during the probe trial. Unexpectedly, a left

lateral injury produced impairments compared to sham controls in both acquisition and expression of memory in the Reversal task. In contrast, the right lateral injury did not differ from sham controls in either measure in this task. Motor behavior was also differentially affected by left and right hemisphere injury, with right lateral injury mice displaying neurological motor deficits for 7 days post-injury in the NSS, and up to 14 days in the Rotarod, compared with a left lateral injury of only 1 day in both assays. In conclusion, differences in Morris water maze task-related demands revealed lateralization of TBI-induced cognitive deficits in mice. The results of the present study suggest that TBI may induce hemisphere dependent functional deficits in mice, consistent with CA3 cellular asymmetries previously noted by others.

Disclosures: **L.D. O'Brien:** None. **T.M. Reeves:** None. **A.J. Morales:** None. **A.H. Lichtman:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

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Title: Lesion-induced brain plasticity: massive increase in neuron number in the monkey amygdala following early or late hippocampal lesion

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Abstract: Focal brain lesions have been shown to trigger adaptive neuronal plasticity in other, sometimes distant, brain areas. Here, we investigated the effects of early and late hippocampal lesions on the fate of a population of Bcl-2-positive, immature neurons found in the ventral part

of the primate amygdala, mainly in the paralaminar nucleus. Four groups of adult rhesus monkeys were studied: 7 unoperated controls, 8 early-lesioned monkeys that received complete bilateral hippocampal lesion soon after birth, 6 late-lesioned monkeys that received complete bilateral hippocampal lesion in adulthood, and 2 lesioned monkeys with limited bilateral hippocampal damage that occurred naturally. First, confocal analyses confirmed that the immature neurons present in the ventral amygdala express both the neuron-specific marker NeuN and the anti-apoptotic marker Bcl-2. Second, using Nissl-stained sections, we performed stereological analyses of the numbers of mature and immature neurons in the paralaminar nucleus, and the adjacent lateral and basal nuclei of the amygdala. Compared to controls, we found that: (1) the number of mature neurons was larger in the paralaminar nucleus of early-, late- and naturally-lesioned monkeys; (2) the number of mature neurons was larger in the lateral nucleus of early- and naturally-lesioned monkeys; (3) the number of mature neurons was larger in the basal nucleus of early-lesioned monkeys. In controls, these three nuclei combined contained 3.7 million mature neurons; we found 5.3 million neurons in early-lesioned monkeys (+43%), 4.0 million neurons in late-lesioned monkeys (+8%) and 5.0 million neurons in naturally-lesioned monkeys (+35%). Interestingly, compared to controls, the number of immature neurons contained in the paralaminar nucleus was higher in early-lesioned monkeys (+38%) and lower in late-lesioned monkeys (-27%). These observations suggest that early hippocampal lesion is followed by (1) the maturation of immature neurons present in the paralaminar nucleus and their integration in the lateral, basal and paralaminar nuclei of the amygdala; and (2) the replacement of a large number of these immature neurons by new cells that possibly migrate from the ventricular zone. In contrast, late hippocampal lesion is followed by the maturation of immature neurons and their integration in the paralaminar nucleus; but it is not accompanied by a replacement of this population of immature neurons. These findings reveal a new mechanism of lesion-induced brain plasticity, characterized by the maturation of immature neurons and their integration in a brain structure interconnected with, yet clearly distinct from the lesioned area.

Disclosures: **L.J. Chareyron:** None. **D.G. Amaral:** None. **P. Lavenex:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

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Topic: C.10. Trauma

Support: NASA Grant NNX13AD94G

Title: Head-down tilt as a model for intracranial hypertension during spaceflight

Authors: *C. A. FULLER, H. GOMPF, E. L. ROBINSON, T. M. HOBAN-HIGGINS;
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Abstract: Introduction: One of the responses to exposure to the microgravity spaceflight environment is a pronounced cephalic fluid shift. Recent evidence documents ocular changes in astronauts following long-duration missions including optic disc edema, globe flattening, choroidal folds and hyperopic shifts. These changes are hypothesized to result from this cephalic fluid shift, as these ocular effects are similar to those seen in terrestrial Idiopathic Intracranial Hypertension (IIH). Long duration missions are currently being undertaken on the International Space Station (ISS) and planning for manned missions to Mars has begun. This project aims to simulate the long-term microgravity cephalic fluid shift using a well-established terrestrial rat model with the objective of establishing a ground-based model for studying CNS changes observed in space. Methods: We are using the rat hindlimb suspension (HLS) model, functionally equivalent to human head-down bedrest (a human terrestrial model of cephalic fluid shift), to examine the relationship between cephalic fluid shifts and the regulation of intracranial pressure (ICP) as well as visual system structure and function. Rats are suspended for 90 days, which is roughly equivalent to the proportion of the lifespan astronauts would dedicate to an exploration class mission, followed by 90 days of observation during recovery in a vivarium cage. All HLS subjects have age-matched cage controls. All animals have ad lib access to food and water. A 12:12 LD cycle is present. Finally, we are also investigating the role of gender and age in these responses. Data collected include ICP, electroencephalogram (EEG) and body temperature (by telemetry) and repeated MRI during HLS and recovery. Brains will be collected for histology from a subset of animals after 90 days of HLS. The study includes three cohorts: young adult male, young adult female, and older middle-age adult males, roughly equivalent in age to most mission-ready astronauts. Results and Conclusions: Results to date indicate a pronounced and sustained increase in ICP during early HLS. This will form the basis for examining anatomical and indications of functional changes found in the MRI images and histology.

Disclosures: C.A. Fuller: None. **H. Gompf:** None. **E.L. Robinson:** None. **T.M. Hoban-Higgins:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

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Office of the Dean, Univ. of Tennessee Health Sci. Ctr. (AJR)

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College of Pharmacy, Univ. of Tennessee Health Sci. Ctr. (BMM)

Methodist Hospitals Endowed Professorship in Neuroscience (AJR)

Title: A mouse model of blast-induced mild traumatic brain injury that yields behavioral deficits and neuron injury is associated with neuroinflammation in the forebrain

Authors: *B. T. WRIGHT, Y. GAO, S. A. HELDT, W. BU, Y. DENG, N. DEL MAR, M. G. HONIG, A. J. REINER;
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Abstract: Mild traumatic brain injury (mTBI) is a common occurrence in combat as well as sports and vehicular accidents. Many mTBI sufferers display persistent neuropsychiatric symptoms that overlap with post-traumatic stress disorder (PTSD), including heightened fearfulness and anxiety - functions normally ascribed to the amygdala. Using a closed-head, high pressure air blast model of mTBI in mice (Heldt et al, Frontiers Neurol, 2014), we have demonstrated the presence of diffuse axonal injury (DAI) in brain, a signature pathology in mild TBI, as well as more recent evidence of neuron loss (Bu et al., SFN 2015). Furthermore, blasted mice showed neuropsychiatric deficits including fearfulness and anxiety (Heldt et al, Frontiers Neurol, 2014). In the present study, we examined neurochemical changes in the brain after mTBI caused by left side focal cranial blast. As a microglial inflammatory response is thought to occur after brain trauma and to worsen the outcome, we assessed the expression of inflammatory markers, using quantitative RT-PCR in forebrain tissue samples taken from mice exposed to blast mTBI versus non-blasted sham controls. Our results indicate that microglial and inflammatory markers are increased in the left forebrain in blasted mice. For example, cannabinoid receptor type-2 (CB2) message appeared to be elevated at 6 hours post blast. As CB2 expression in brain is largely limited to microglia, the elevated CB2 is suggestive of post-blast microglial activation. Immunolabeling for IBA1 showed that microglia are activated in many brain regions, particularly in fiber tracts in which axonal damage was observed, such as the optic tract, pyramidal tract, lateral lemniscus, and medial lemniscus. In forebrain, quantitative RT-PCR showed that interleukin1-beta, tumor necrosis factor-alpha and interleukin-10 expression were elevated at 6 - 24 hours after blast, and tumor necrosis factor-alpha remained elevated at 48 hours. The increase in expression of cytokines we observed indicates a brain inflammatory response in our TBI model. This inflammatory response could play a role in worsening the severity of the outcome after blast. Consistent with this, we have found that a CB2

receptor inverse agonist that mitigates the microglial inflammatory response reduces deficits and brain pathology in our mTBI model (Reiner et al, Int J Molec Sci 2015).

Disclosures: **B.T. Wright:** None. **Y. Gao:** None. **S.A. Heldt:** None. **W. Bu:** None. **Y. Deng:** None. **N. Del Mar:** None. **M.G. Honig:** None. **A.J. Reiner:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

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Support: University of Washington CHDD Animal Behavior Core

University of Washington CHDD Brain Imaging Core

Alzheimer's Association

Title: Radial water tread maze distinguishes cognitive deficits in mice with traumatic brain injury and Alzheimer's disease

Authors: *M. M. CLINE[1], J. YUMUL[2], L. HYSA[2], D. MURRA[2], E. BRIM[2], G. GARWIN[1], W. C. LADIGES[3], S. MINOSHIMA[4], D. J. CROSS[1];
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Abstract: Introduction: Interspecies differences create challenges when using cognition tests designed for rats to assess mouse models. The most common such test, the Morris water maze, capitalizes on rat swimming ability by employing a forced-swim paradigm: a task aversive and difficult for mice. Mouse models of traumatic brain injury (TBI) and Alzheimer's disease (AD) are susceptible to compounding factors such as impaired motor function and stress-induced non-compliance during forced-swim testing. We evaluated the ability of a Radial Water Tread (RWT) maze, designed specifically for mice and requiring no swimming, to distinguish between Sham and TBI, as well as AD and wild type (WT) models. Methods: Male C57BL/6J mice 10-weeks old (n=29) were randomly assigned to receive craniotomy (Sham, n=14) or craniotomy plus controlled-cortical impact (Impact One, Leica Biosystems) surgery (TBI, n=15). RWT maze was acquired on a subset of mice at 11 days (early time point; Sham=8, TBI=9) and 35 days (late time point; Sham=6, TBI=6) post-injury. For our AD model, male 3xTg-AD mice (n=4) aged 56.7 weeks were tested and compared with age matched WT controls (n=5). Briefly, the testing

apparatus consisted of a 32-inch tub with one escape and nine decoy holes spaced evenly around the device, and filled with roughly 1½ inch of 12-16 °C water. Five unique visual cues lined the apparatus. RWT testing consisted of 3 trials/day for 4 days, short-term memory test at day 5, and long-term memory at day 12. Results: RWT maze successfully distinguished between Sham and TBI 11 days post-injury on both short and long-term testing days (day 5; 20.80±14.68s vs. 48.58±37.65s & day 12; 10.85±8.09s vs. 70.54±57.78s for Sham and TBI respectively), and long-term testing 35 days post-injury (19.88±20.11s vs. 61.06±31.96s; $p<0.05$). In our AD model, RWT maze successfully distinguished between AD and WT at both short and long-term memory tests (day 5; 143.83±43.02s vs. 64.2±59.84s & day 12; 138.33±52.05s vs. 33.4±49.11s), as well as 3 out of the 4 training days. Conclusion: The RWT maze was designed to capitalize on mouse instinctual behavior and presents a novel alternative to rat-biased cognition tests. Our results lend validation to the use of this apparatus in both TBI and AD mouse models.

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Poster

688. Traumatic Brain Injury: Animal Models II

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Topic: C.10. Trauma

Support: DOD W81XWH-13-2-0095

VA 1IO1RX001774

Title: The role of tau and other pathologies in an animal model of repetitive mild traumatic brain injury

Authors: ***B. C. MOUZON**[1,2,3], C. BACHMEIER[1,3], J. OLUBUNMI[1,3], S. FERGUSON[1,2], V. MATHURA[1,3], C. LYNCH[1,3], R. WILLIAMS[4], E. MUFSON[5], F. CRAWFORD[1,3,2];
[1]The Roskamp Inst., Sarasota, FL; [2]James A. Haley Veterans' Hosp., Tampa, FL; [3]The Open Univ., Milton Keynes, United Kingdom; [4]RTI Intl., Research Triangle Park, NC; [5]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: The Chronic Effects of Neurotrauma Consortium or CENC is dedicated to understanding the chronic sequelae associated with neurotrauma, primarily focused on mild TBI (mTBI)/concussion incurred by U.S. service personnel. However, little is known about the

timeline and sequence in which tau is processed following TBI, nor about the relationship with other TBI-dependent neuropathologies such as neuroinflammation and cerebrovascular changes. This study is evaluating tau alterations and accompanying neuropathologies over time after r-mTBI in hTau transgenic mice. hTau mice aged either 8-12 weeks will receive either r-mTBI or r-sham in order to control for effects of repeated anesthesia. Mice will be euthanized for neuropathological, genomic and biochemical analyses at 24hrs, 5, 10 and 15 days, 3, 6 and 12 months after last mTBI/anesthesia. For the 15 day and 3, 6 and 12 month time points post injury mice will undergo a battery of neurobehavioral test in the 2 weeks immediately prior to euthanasia. The entire paradigm is being replicated in hTau mice aged 12 months at the time of injury, to study the effects of age at time of injury. r-mTBI in the young cohort shows learning impairment post injury that progressively worsens from 2 weeks to 12 months. To date, Tau IHC and ELISA results suggest that r-mTBI is associated with a transient injury dependent increase in p-tau accumulation with greater dendritic and membranous staining in the cerebral cortex beneath the impact site without neurofibrillary tangles. While a trend for an increase in aggregated tau at 12 months post injury was observed, r-mTBI was not associated with elevated brain levels of abnormal soluble tau phosphorylation. This study is ongoing and will take several years to complete; our previous data suggest that neuroinflammatory pathology is key in this model and that TBI-dependent tau pathology will be evident in the older mouse models where tau pathology already exists.

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Poster

688. Traumatic Brain Injury: Animal Models II

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Topic: C.10. Trauma

Support: NIH K08NS069815

Title: Impaired fatty acid oxidation after traumatic brain injury exaggerates the glial response and promotes cell death

Authors: *J. N. JERNBERG[1], C. BOWMAN[2], M. WOLFGANG[2], J. LEE[2], S. SCAFIDI[1]; [1]Anesthesiol. and Critical Care Med., [2]Biol. Chem., Johns Hopkins, Baltimore, MD

Abstract: Traumatic brain injury (TBI) is a leading cause of permanent life-long disability and is characterized by deficits in cognition, attention and sensory-motor integration. Impaired oxidative glucose metabolism following TBI further contributes to cell death. The role of fatty acid (FA) oxidation after TBI, however, is unknown. Mitochondrial β-oxidation of fatty acids supports energy production and metabolic homeostasis, especially during the periods of fasting and stress. To be oxidized in mitochondria, fatty acids must be converted to acyl-carnitine esters by transfer of acyl groups to l-carnitine. This reaction is catalyzed by Carnitine Palmitoyl Transferases (CPT1a and CPT2), which are located on the outer and inner mitochondrial membranes respectively. Only then are acyl-carnitines transported into mitochondria to support oxidative metabolism. This study is the first to explore the role of fatty acid oxidation after TBI. To determine the requirement of mitochondrial fatty acid β-oxidation after TBI, Cpt2 floxed mice to Nestin-Cre mice (Cpt2N-/-), which cannot oxidize fatty acids within the brain, were generated. TBI was administered using a controlled cortical impact to the left parietal cortex and tissue was collected for histology 7 days post-injury. Using immunofluorescence, we determined that astrocytes are the only cells expressing CPT1a and CPT2 in WT mice, thus the only cells able to use fatty acids for energy and metabolism. CPT1a also colocalizes with the astrocytic marker GFAP in CPT2 KO mice. Iba1 expression was increased and microglia demonstrated a more activated M1 phenotype in CPT2 KO mice relative to WT, particularly within the hippocampus. GFAP was also increased in KO relative to WT after TBI. Lesion size was greater in CPT2 KO than WT. Fatty acid oxidation in the cortex and hippocampus was measured using [1-14C] oleic acid, and oxidation was increased after TBI in the ipsilateral hippocampus but not in the cortex. This study provides strong evidence that 1) astrocytes selectively oxidize fatty acids for energy and metabolism 2) brain-specific knockout mice unable to utilize long chain fatty acids exhibit increased astrocytic and microglial activation after TBI, as well as 3) increased lesion size relative to wild-type controls. These results indicate that fatty acid oxidative metabolism may be beneficial in promoting neuronal survival and reducing TBI-induced glial activation.

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Poster

688. Traumatic Brain Injury: Animal Models II

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Title: Human chorionic gonadotropin treatment improves cognitive and motor performance following focal penetrating traumatic brain injury in adult male rats

Authors: *R. I. GEDDES, K. HAYASHI, M. WEHBER, R. A. RAUH, S. V. MEETHAL, C. S. ATWOOD;
Emergency Med., Univ. of Wisconsin at Madison, Madison, WI

Abstract: Traumatic brain injury (TBI) is a major US public health issue with ~270,000 people experiencing a moderate or severe TBI each year that results in chronic deficits to cognitive and motor function. Unfortunately, to date there is no satisfactory therapeutic intervention for TBI. The spontaneous, although often incomplete, cognitive recovery that occurs following TBI is thought to involve neuroregeneration that includes neurogenesis. Neurogenesis during embryogenesis and adulthood is driven by the key neurohormones gonadotropins and sex steroids. However, the stress from a TBI has been shown to markedly suppress circulating gonadotropin and sex steroid concentrations. In this study, we tested whether chronic administration of human chorionic gonadotropin (hCG), a neurodevelopmental gonadotropin, would promote neurogenesis in the adult brain thereby acting to enhance functional recovery. Male Sprague Dawley rats at 5 months of age were subjected to sham surgery (craniectomy) or a focal penetrating TBI using a controlled cortical impact (CCI) device (Hatterras, Inc.), n = 5-7 rats per group. hCG (200 mIU/kg) was administered intramuscularly at 1 h post-surgery and every 48 h thereafter for 28 d. Behavioral analyses were performed and plasma sampled before and 24 h post-CCI and weekly thereafter for assessment of cognitive and motor function, and circulating sex hormones, respectively. When compared to CCI-injured vehicle-treated rats, hCG-treatment (1) significantly increased the latency to fall off the rotarod in CCI-injured male rats by ~30 seconds or 20% (170 seconds vs. 135 seconds) over repeated testing, $F(25,135) = 3.994$, $p < .0001$) and (2) reduced the latency to reach the hidden Morris water maze platform by ~12 seconds or 34% (23 seconds vs. 35 seconds), $F(35,189) = 3.722$, $p < .0001$), while having (3) no effect on CCI-induced decrease in the distance travelled in an open field tasks (p's $> .05$) or (4) the surface area of the cortical contusion site ($p > .05$). A more in-depth analysis of lesion volume, as well as plasma hormones, cell death, inflammation and hCG-induced neurogenesis are currently underway. Importantly, non-surgical control (NSC) and sham-injured animals given hCG did not perform different from vehicle-treated NSC and sham-injured animals on any measures. We speculate that hCG mediates improvement in functional performance via decreasing inflammation and edema while promoting neurogenesis. hCG, a readily available neurohormone, may provide a physiological means of promoting functional improvement in the recovery from TBI.

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Poster

688. Traumatic Brain Injury: Animal Models II

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Support: VA IK2RX001479

VA IRX001097A

Title: Neuropathological alterations in the hilus after diffuse brain injury

Authors: M. R. GROVOLA[1,2], J. P. HARRIS[1,2], D. CULLEN[1,2], *J. A. WOLF[2,1]; [1]Neurosurg., Philadelphia Veterans Affairs Med. Ctr., Philadelphia, PA; [2]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Each year in the United States, over 1.5 million people experience mild traumatic brain injury (mTBI) and recent studies suggest that mTBI is associated with long term physical and mental health problems. Functional and structural changes induced by mTBI may occur in the hippocampus, with the hilar region showing particular vulnerability in other models. Mossy cells, one of the predominant cells in the hilar region, have been a focus of attention due to their potential role in post-TBI hyperexcitability. The objective of the current study was to assess mossy cell pathology and microglial changes after a range of mTBI injuries. 5-month-old Yucatan swine were induced with mTBI using a coronal rotational head injury model of diffuse brain injury. Swine were subjected to sham conditions or coronal plane rotations at peak velocities of 180, 220, or 260 rad/sec. Neuropathological changes in the hilar region of the hippocampus were assessed at 6 hour, 7 day, and 14 day time points. Whole coronal sections of tissue were labeled with antibodies for MAP2, a microtubule associated protein, Synapsin, a pre-synaptic protein, and IBA1, a calcium binding protein used as a microglia marker. Using confocal microscopy, we measured the mossy cell somal area using MAP2, and visualized synaptic and microglial locations using Synapsin and IBA1 respectively. We observed the prevalence of microglia migrating within the synaptic clouds and their processes engulfing mossy cells in injured specimens, suggesting that microglia are recruited post-injury to remove disrupted synaptic components around mossy cells. Additionally, quantitative analysis of mossy cell somal area has demonstrated an increase in somal area at 7 day and 14 day time points post-

injury compared to sham and 6 hour time points. This alteration of mossy cell area paired with the increase of microglia processes around the cell demonstrates the vulnerability of hilar mossy cells after time points post-mTBI. Moreover, mTBI-induced hilar mossy cell pathology may lead to aberrant activity. This activity may effect dentate granule cells along the entire dorsoventral axis and enhance perforant path inputs.

Disclosures: **M.R. Grovola:** None. **J.P. Harris:** None. **D. Cullen:** None. **J.A. Wolf:** None.

Poster

688. Traumatic Brain Injury: Animal Models II

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Phoenix VA Health Care System

PCH Mission Support Funds

Science Foundation Arizona

Title: Diffuse traumatic brain injury affects chronic corticosterone levels and alters neuron morphology in the paraventricular nucleus

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Abstract: As many as 20-55% of patients with a history of traumatic brain injury (TBI) experience chronic endocrine dysfunction, leading to impaired quality of life, impeded rehabilitation efforts, and lowered life expectancy. Endocrine dysfunction after TBI is thought to result from acceleration-deceleration forces to the brain within the skull, creating enduring hypothalamic and pituitary neuropathology, and subsequent hypothalamic-pituitary (HP)-axis

dysfunction. These experiments were designed to test the hypothesis that a single diffuse TBI results in chronic dysfunction of corticosterone (CORT), a glucocorticoid released in response to stress, with evidence of structural damage to the HP-axis. We used a rodent model (adult, male Sprague-Dawley rats) of diffuse TBI induced by midline fluid percussion (mFP). At 2 months post-injury, circulating levels of CORT were evaluated at rest, under restraint stress and in response to dexamethasone, a synthetic glucocorticoid commonly used to test HP-axis regulation. Further, we assessed changes in injury-induced neuron morphology (Golgi stain) and neuropathology (silver stain) in the paraventricular nucleus (PVN) of the hypothalamus. Resting plasma CORT levels were decreased by ~60% at 2 months post-injury and the dynamic release of CORT in response to restraint stress was significantly reduced in injured animals. TBI also altered complexity of neuron processes in the PVN over time. In order to determine when changes in CORT dysfunction were first detectable, a second cohort of animals containing sham, mild and moderate injured animals had repeated blood draws and restraint stress over two months. Resting CORT levels were significantly decreased in all test groups compared to baseline levels by 14 days post-injury ($p<0.05$), indicating that repeated exposure to stress masks the injury effect. Results provide evidence that a single moderate diffuse TBI leads to hormonal and structural changes, as it pertains to the HP-adrenal axis, which can contribute to the persistence of endocrine dysfunction.

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Poster

688. Traumatic Brain Injury: Animal Models II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 688.29/J41

Topic: C.10. Trauma

Title: The effect of prolonged sleep deprivation on cognitive and behavioral outcomes following mild traumatic brain injury

Authors: R. CHIARIELLO[1], A. SHAH[1], N. WILKINS[1], M. BUDDE[1], C. OLSEN[2], F. A. PINTAR[1,3], D. THOMAS[4], *B. D. STEMPER[1,3];
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Abstract: Mild traumatic brain injury has become an increasing concern in military personnel and athletes. There may be clinical uncertainty regarding whether or not to allow individuals to rest immediately following an exposure. In some cases, sleep may be interrupted for extended periods during deployment. The objective of this study was to delineate the confounding effects of sleep deprivation and TBI. Anesthetized female Sprague-Dawley rats were exposed to head rotational acceleration at a peak magnitude of 334 krad/s2 and pulse duration of 3.7 ms. Sleep-deprived control rats were exposed to a sham procedure that was identical to the injury protocol without head rotational acceleration. Immediately following exposure, animals (n=9 injured (SDTBI) and n=8 controls (SDC)) were placed on an analog orbital shaker for 48 hours. Repetitive on/off cycling of the shaker, which was set at 100 rpm, was controlled by a timer on a 120 second cycle (30s on, 90s off). An injured group of non-sleep-deprived animals (n=8, NSDTBI) was used for comparison. Cognitive and behavioral assessments including Morris Water Maze (MWM) and Elevated Plus Maze (EPM) were conducted on days 3-7 post injury. The SDTBI and SDC groups spent more time in the open arms of the EPM than the NSDTBI group (+29% and +33%, respectively), indicating decreased inhibition for rats receiving the sleep deprivation protocol. The total distance traveled was also greater for the SDTBI (+17%) and SDC (+16%) groups than for the NSDTBI group. The SDTBI group demonstrated an increased number of unsuccessful trials in the MWM (23% and 51% greater than the NSDTBI and SDC groups, respectively). A trend approaching significance (p=0.057) was seen in the latency to find the platform in the MWM. The SDTBI group took longer to reach the platform (24 seconds) than both the SDC (19 seconds) and NSDTBI (20 seconds) groups. These findings demonstrate a complex relationship between prolonged sleep deprivation and TBI, with sleep deprivation dominating acute changes in emotionality and coupled sleep deprivation with TBI leading to a dramatic acute increase in cognitive deficit. This finding is significant as our prior work highlighted significantly increased acute changes in emotionality for NSDTBI compared to controls that did not receive TBI or SD. However, cognitive testing demonstrated a different trend with SD plus TBI producing significant deficits compared to SD or TBI alone. Assessment of the chronic effects of sleep deprivation and TBI is an ongoing focus of these studies.

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Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.01/J42

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

Support: CNPq Grant 307064/2013-1

CNPq/INCT/INPeTAm Grant 573695/2008-3

PRONEX/FAPERGS/CNPq Grant 10/0044-3

Title: Gamma-decanolactone effects on pilocarpine-mediated seizure in mice and oxidative stress parameters in N9 Cells

Authors: *P. PEREIRA[1], P. PFLÜGER[1], V. COELHO[1], L. DA SILVA[1], C. VIEIRA[1], N. BERWIG[2], R. STAUB[2], C. VIAU[2], J. SAFFI[2];
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Abstract: Gamma-decanolactone (GD) is a monoterpenic compound that has demonstrated dose-dependent hypnotic, anticonvulsant, and hypothermic activities. In previous studies, *in vivo* and *ex vivo*, it has shown a protective effect on seizures induced by pentylenetetrazol and it has been able to inhibit the glutamate binding in cortex homogenate. The goal of this study was to evaluate the effect of GD on pilocarpine-induced seizures in mice and on oxidative stress parameters in N9 cell. To evaluate the effect of GD on pilocarpine-induced seizures, mice received the controls or GD (100 and 300 mg/kg), 30 min before the pilocarpine injection (250 mg/kg, i.p.). The latency to the first seizure and the occurrence of clonic seizure ≥ 3 seconds were observed during 1 h. The murine N9 microglial cell line was obtained by immortalization of embryonic brain cultures with 3RV retrovirus carrying an activated v-myc oncogene and was grown in RPMI. Cells (1 × 104 cells/mL) were seeded on 6-well tissue culture plates and grown for 1 day up to 70-80% confluence before treatment with the test compound. In all tests, N9 microglial cells were pretreated with GD (10, 50 and 100μM) for 20 hours and exposed to LPS (1μ/mL) for 4 h. Cell viability was measured by Trypan blue exclusion assay (TB). Reactive oxygen species (ROS) detection and quantification of cleaved caspase-9 were performed by flow cytometric analysis. After the treatment, the total protein was obtained using RIPA buffer and Western blot analysis was performed using anti-iNOS antibodies. Antigens were detected using the chemiluminescence technique. Image analysis was performed using an analysis software. The effect of GD on DNA was evaluated by Comet Assay. Data were analyzed by Fisher Test or ANOVA followed by Tukey or Student-Newman-Keuls. The highest dose of GD significantly increased the latency to onset of first seizure; however, the percentage of seizure was not significantly reduced. GD was not cytotoxic at the doses tested in N9 cells. At 50 and 100 μM doses GD inhibited the formation of ROS (DCF). It reduced DNA damage and the expression of caspase 9, an important pathway which regulates apoptosis. Moreover, GD decreased the expression of iNOS protein, one of the factors involved in inflammation processes. To conclude, the results indicated the highest GD dose (300 mg/kg) was able to alter parameters associated with pilocarpine-mediated seizures in mice. The results also suggested that GD at 50 and 100 μM

had a protective effect against the harmful consequences caused by LPS-induced oxidative stress, inflammation, and cell damage.

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Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.02/J43

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

Title: An animal model of brain reversible vasogenic edema

Authors: *S. KIMURA[1], Y. YANG[2], J. THOMPSON[2], Y. YANG[3], L. SILLERUD[2], G. ROSENBERG[2];
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Abstract: [Introduction] Brain vasogenic edema, defined by the disruption of the blood-brain barrier, is a fundamental and clinically common pathological condition in several neurological diseases. Posterior Reversible Encephalopathy Syndrome (PRES) is unique in that it is one of the few diseases which presents only with vasogenic edema and whose clinical course is usually reversible. Although vasogenic edema is heterogeneous in prognosis, the present paradigm could not determine the reversibility. [Methods] SHR-SP rats were treated with high-dose cyclosporine A to induce encephalopathy mimicking PRES. The recovery from the encephalopathy was achieved by the cessation of cyclosporine A. The extent and recovery of neurological symptoms and brain lesion were monitored by neurological score, behavioral tests, and MRI. Blood-brain barrier leakage was histologically confirmed. [Results] Rodent PRES model showed neurological symptoms and worsening scores in behavior tests, whose manifestations disappeared after the recovery. Brain lesions of PRES model revealed IgG leakage, suggesting the presence of vasogenic edema. This PRES model presented some characteristic changes in FA and ADC signals. [Conclusion] These results suggest that our PRES model represents reversible vasogenic edema. Fluid leakage in vasogenic edema may be the cause of FA change in PRES model.

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Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.03/J44

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

Title: Differential effects of seed extracts of citrus fruits against hydrogen peroxide-induced toxicity in SH-SY5Y cells

Authors: *K. SUEN[1], W. TANG[1], P. HAU[1], H. MA[1], P. CHOW[1], Y. HUNG[1], P. SHI[1], W. CHAU[1], W. CHAN[1], C. CHANG[2];
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Abstract: Some plant seed extracts have been reported to offer neuroprotection (Okada and Okada 2013; Yang et. al. 2012). The neuroprotective effects of seed extracts from *Citrus limon* (Common name: Lemon) and *Citrus margarita* (Common name: Kumquat) have not yet been studied. It is believed that oxidative stress is most likely to act in some neurodegenerative processes. Agents that have antioxidant effects may possess neuroprotective functions. In the present study, the neuroprotective effects of seed extracts from two types of citrus fruits which are *Citrus limon* and *Citrus margarita* are examined. The extracts were prepared by dissolving the powder of ground fresh seeds in DMSO or ethanol and insolubles were filtered out by 0.22 μm membrane filter. Non-differentiated SH-SY5Y cells were pre-treated with either *Citrus limon* or *Citrus margarita*'s seed extract for 24 hours in DMEM supplemented with 2% fetal bovine serum. Following the removal of the seed extract, SH-SY5Y cells were treated with 500 μM of hydrogen peroxide for 24 hours in DMEM with 2% fetal bovine serum. Results indicated that the seed extract of *Citrus margarita* can significantly reduce toxicity induced by hydrogen peroxide. Yet, *Citrus limon*'s seed extract did not show attenuation of hydrogen peroxide-induced cytotoxicity. This implicates that seed extracts from different types of citrus fruits may have different effects against toxicity induced by hydrogen peroxide. The present findings suggest that *Citrus margarita*'s seed extract may offer neuroprotection.

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Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

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Program#/Poster#: 689.04/J45

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

Support: MOST 103-2320-B-182A-011-MY3

CMRPG8B0573

Title: Activation of glp-1 receptors up-regulates apurinic/apyrimidinic endonuclease 1 protects neurons against oxidative dna damage

Authors: *J.-L. YANG, Y.-T. LIN, P.-C. CHUANG, S.-D. CHEN;
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Abstract: Neurons are terminally-differentiated non- proliferating cells with a high risk for DNA damage because of their highly metabolic activity. Therefore, DNA repair is important for maintaining DNA integrity and neuronal survival. However, the impaired DNA repair capacity is suggested to play roles in normal aging and aging-associated neurodegenerative disorders. Base excision repair (BER) is commonly suggested being the major mechanism that repairs oxidative lesions, alkylated DNA bases, single-strand breaks, and abasic sites in nuclear and mitochondrial DNA. Therefore, elevating BER efficiency may be one of important mechanisms maintaining neuronal function and survival. Results of the present study has shown that activation of Glucagon-like peptide-1(GLP-1) receptors up-regulates expression of apurinic/apyrimidinic endonuclease 1 (APE1), an important enzyme of BER, via PI3K-Akt signaling pathway in rat primary cortical neurons. We also have observed that treatment of GLP-1 and its analogue rescuing cortical neurons against menadione induced oxidative insults, but the inhibitor of APE1compromised the neuron protective function of GLP-1. Many recent studies suggested that function of GLP-1 is not only decreasing blood sugar, but also acts as a neurotrophic factor and plays a role in neuronal survival, neurite outgrowth, and protecting synaptic plasticity and memory formation from effects of β-amyloid. Our study suggested that GLP-1 up-regulated APE1 enhances neuronal viability via increasing efficiency of BER. Therefore, enhancement of DNA repair efficiency is a potential strategy for prevention or therapeutic intervention in ischemic stroke and neurodegenerative diseases.

Disclosures: **J. Yang:** None. **Y. Lin:** None. **P. Chuang:** None. **S. Chen:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.05/J46

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

Support: Nipissing University

Title: Social isolation alters the toxic effects of ethanol in planarians

Authors: T. MCCHARLES, B. LOVELL, N. LANDRY, A. STILLAR, A. WEEKS, *M. J. SAARI;
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Abstract: In order to examine the potential interaction between low doses of alcohol and social isolation, planarians (Dugesia dorotocephala), were placed into isolation and exposed to ethanol (EtOH) while motor behaviours were examined. Control, non-isolated planarians were held in groups of ten. The planarians were placed in one of four conditions of EtOH: vehicle (30ml dechlorinated H2O), 1.0%, 1.5%, or 2.0%. These concentrations were chosen based on a pilot study to confirm EtOH thresholds that cause complete immobility of the planarians. Behavioural testing was carried out in a glass dish (10 cm diameter) which was placed on a 1cm x 1cm grid with a 5.35cm diameter interior circle drawn to designate a central field. Each subject was placed into dechlorinated H2O for five minutes, before being transferred to their EtOH condition. After the five minute exposure to the EtOH solution, the planarians were transferred back into the water bath. Motor behaviours, such as grid crossings, environmental surveys (raising the anterior portion of the body), and time spent in the peripheral or central regions of the Open Field were recorded, minute by minute, for each planarian. The results indicated that exposure to EtOH appeared to reduce motor behaviour in a dose dependent manner. A significant interaction was found between social condition and EtOH concentration, with the isolated group showing differences in activity between the highest concentrations of EtOH and the vehicle. Comparable effects were noted for the environmental survey measure. We suggest that social effects may ameliorate the behavioural sensitivity of planaria to toxins, such as EtOH. (Supported by Nipissing University).

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Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.06/J47

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

Title: Development and utilisation of a high content assay to identify putative small molecule modulators of expression and trafficking of the neuronal survival factor NMNAT2

Authors: J. FRANCIS[1], *D. F. FISCHER[2], G. SMITH[1], G. MCALLISTER[1], W. J. RAY[3], M. GECK DO[3], D. VENKITARAMANI[3];
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Abstract: The NAD-synthetic enzyme NMNAT2 was recently identified as an endogenous neuronal survival factor acting as a chaperone for neuronal maintenance and protection (1,6). Depletion of NMNAT2 is reported to result in neuronal degeneration in the absence of injury (2), whilst overexpression *in vitro* has been demonstrated to have a protective effect on Wallerian degeneration that is dependent on NMNAT2 enzymatic activity (3). Overexpression of NMNAT2 has also been shown to have beneficial effects on axonal loss *in vivo* in the P301L mouse model of human tauopathy (4). These data suggest that a small molecule able to increase relative levels of cytosolic NMNAT2, for example by increasing the stability/half-life of the enzyme, could have therapeutic potential in a number of neurodegenerative diseases. Here we describe the development of a high content assay for the identification of small molecule modulators of NMNAT2 stability. Using a stable cell line expressing Flag tagged NMNAT2 the half-life of the enzyme was found to be ~ 100 minutes, suggesting this enzyme is fairly labile. Therefor in a pilot screen using a subset of 2000 SoftFocus® kinase targeting compounds were screened for three hours after which the NMNAT2 protein level and subcellular localization was assessed using automated high content imaging. Interestingly, a number of compounds appeared to up-regulate NMNAT2 levels with a 1 % hit rate. Co-localization studies revealed accumulation of NMNAT2 within the trans-golgi network in cells treated with DMSO alone over this time course. However, after compound treatment, modulators of NMNAT2 expression fell into two distinct phenotypic classes; those that up-regulated expression of NMNAT2 in the Golgi and those that appear to promote trafficking of NMNAT2 from the Golgi resulting in an increase of cytosolic NMNAT2 levels. The latter group represent an interesting class of compounds for validation of the role of NMNAT2 localization in its regulation and turnover; this is of particular interest as it has recently been postulated that the cytosolic form of NMNAT2 has an increased half life and is a key determinant in its role in the prevention of neurite degeneration (4). On the basis of this pilot screen, a larger unbiased HTS campaign has been initiated. 1. Zhai.R, Bellen.H. *et. al.* 452, 887-91, Nature. (2008) 2. Gilly.j and M.Coleman PLoS Biol. e1000300 (2010) 3. Yan.T, Feng.Y, Zheng.J, Ge.X, Zhang.Y *et. al* Neurochem Int. 56:101-106. (2010) 4.

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Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.07/J48

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

Support: MOST 103-2314-B-006 -017-MY3

Title: Insulin receptor substrate -1 protects against neonatal hypoxic-ischemic encephalopathy

Authors: ***Y.-F. TU**[1], H.-I. SHIH[2], C.-C. HUANG[3];
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Abstract: Background Neonatal hypoxic-ischemic (HI) encephalopathy is a major cause of neonatal mortality and of subsequent neurological disabilities among infants who survive it. Insulin receptor substrate -1 (IRS-1) is a ducking protein, which binds to phosphotyrosine residues on insulin-like growth factor-I or insulin receptors, and involves in activation of phosphoinositide3-kinase (PI3K) / Protein kinase B (Akt) pathways. Based on our previous study, we hypothesized that IRS-1 could protect against neonatal HI brain injury. Methods We used *in vivo* HI animal model and *in vitro* oxygen-glucose deprivation (OGD) model to test the effect of IRS-1 after HI injury on neonatal rat brain and cells. IRS-1 was modulated by either adenovirus or anti-sense oligonucleotide/shRNA. The treatment of proposed IRS-1 activator, metformin, was also conducted in both HI models. Results IRS-1 down-regulation reduced p-Akt, increasedp53, worsened blood brain-barrier (BBB) damage, and increased brain infarct volume, whereas IRS-1 over-expression up-regulated p-Akt, decreasedp53, attenuated BBB

damage, and decreased brain injury. *In vitro*, IRS-1 down-regulation aggravated cell death in neurons and endothelial cells, and is associated with decreased p-Akt and increased p53. In contrast, IRS-1 over-expression reduced cell death in endothelial cells with increased p-Akt and decreased p53. For clinical application, we found that metformin can increase IRS-1 in neuronal and endothelial cells. It increased cell viability after OGD, and the metformin-treated rats apparently reduced HI-induced cell death, BBB damage and infarct brain volume. Conclusion IRS-1 can protect against neonatal HI brain injury and is a therapeutic target for developing neuroprotective treatment.

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Poster

689. Neurotoxicity: Protective Mechanisms

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Program#/Poster#: 689.08/K1

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

Support: CNPq

FAPEMIG

Title: Orchestrated activation of the glutamatergic and cannabinoid systems can promote neuroprotection in primary cultured corticostriatal neurons

Authors: *F. M. RIBEIRO, E. M. L. BATISTA, T. H. FERREIRA-VIEIRA, F. A. MOREIRA; Univ. Federal De Minas Gerais, Belo Horizonte, Brazil

Abstract: Endocannabinoids are retrograde lipid regulators that bind and activate CB1 and CB2 receptors, which are linked to the Gi/0 protein. Cannabinoid receptors are present at the pre-synaptic site and their activation can reduce neurotransmitters release. Endocannabinoids can also regulate kinases, including extracellular regulated kinase (ERK), phosphatidylinositol-3-kinase (PI3K) and AKT, as well as transcription factors and ionic channels, having a role in cell survivor, differentiation and plasticity. It has been shown that the activation of the endocannabinoid system can induce neuroprotective effects both in animal models of neurodegenerative diseases and cell cultures. Despite of this, the molecular mechanisms and the signaling pathways involved in endocannabinoid-mediated neuroprotection are still poorly known. Moreover, data published by our research group and others have shown that activation of the metabotropic glutamate receptor 5 (mGluR5) by positive allosteric modulators (PAMs),

including CDPPB, can protect against glutamate-induced excitotoxicity. Since there is a close relationship between the glutamatergic and cannabinoid systems, we decided to investigate a possible link between CB1 and mGluR5 activation in the induction of neuroprotection using primary cultured corticostriatal neurons. To test that, neuronal cell death was assessed in neurons that were incubated with glutamate in the presence or absence of the endocannabinoid potentiating drugs, URB597 and JZL184, and the mGluR5 positive allosteric modulator, CDPPB. URB597, JZL184 or CDPPB led to neuroprotection against glutamate induced neuronal cell death. Surprisingly, the CB1 antagonist, AM251, reversed neuroprotection induced not only by URB597 and JZL184, but also by CDPPB. On the other hand, MPEP, which is an mGluR5 negative allosteric modulator, was also capable of ablating neuroprotection induced by CDPPB, URB597 or JZL184. These data indicate that there is a functional interaction between these two neurotransmitter systems to promote neuroprotection. Interestingly, stimulation of neuronal cultures with CDPPB, URB597 or JZL184, in the presence or absence of glutamate, promoted activation of ERK1/2 and AKT above basal levels. In addition, CDPPB, URB597 or JZL184 decreased intracellular Ca2+ levels induced by glutamate stimulation. Thus, mGluR5 and CB1 receptors seem to activate similar cell signaling pathways leading to neuroprotection.

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Poster

689. Neurotoxicity: Protective Mechanisms

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Program#/Poster#: 689.09/K2

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

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IT 794/13 (JVL), Government of Basque Country

Title: Timed released of cerebrolysin using drug loaded titanate nanospheres improves behavioral functions and reduces brain pathology in Parkinson's disease

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Abstract: Parkinson's disease (PD) induces severe disability in victims and so far no suitable strategies have been developed. Thus, there is an urgent need to explore novel therapeutic strategies to treat PD for the benefit of the mankind. Recently, nanodrug delivery of therapeutic compounds has been shown to induce superior neuroprotective effects than the parent compounds in central nervous system (CNS) diseases. However, there are evidences that mode of nanodelivery e.g., using Poly Lactic-co-Glycolic Acid (PLGA), or TiO2 nanowires have slightly but significantly different effects on their neuroprotective ability in CNS diseases. Thus, there is an urgent need to find our suitable mode of nanodrug delivery to induce maximum neuroprotection in various neurodegenerative diseases e.g., PD, Alzheimer's disease (AD) or Huntington's disease (HD) in model experiments. Previous studies from our laboratory showed that intraperitoneal injections of or 1-metyl-4-fenyl-1,2,3,6-tetrahydropyridin (MPTP, 20 mg/kg) daily within 2-h intervals for 5 days in mice induces PD like symptoms on the 8th day. Thus, in this model, there is a significant decrease in dopamine (DA) and its metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Also immunohistochemical studies showed marked decrease in the number of tyrosine hydroxylase (TH) positive cells in the Substantia Nigra Pars Compacta (SNpc) and striatum (STr). These observations indicate that our model can be used for drug treatment studies in PD. Interestingly, these MPTP treated animals exhibited marked behavioral dysfunctions on the 8th day as evaluated on Rota-Rod performances, walking on an inclined mesh grid as well as their gait analyses. Cerebrolysin (CBL) is a well-balanced composition of several neurotrophic factors and active peptide fragments. Thus, this multimodal drug may have an added value on therapeutic strategies in PD. In present investigations we examined timed release of CBL using titanate nanospheres (TiNS) in treating PD in our mouse model. Our observations show that TiNS-CBL (in a dose of 3 ml/kg, i.v.) given after 2-days of MPTP administration for 5 days resulted in a marked increase in TH-positive cells in the SNpc and STr as compared to normal CBL. Also TiNS-CBL resulted in significantly higher levels of DA, DOPAC and HVA in SNpc and STr on the 8th day as compared to normal CBL. The behavioral functions were also improved significantly in MPTP treated animals that received TiNS-CBL. Tehse observations are the first

to point out that timed release of TiNS-CBL has far more superior neuroprotective effects in PD than normal CBL, not reported earlier.

Disclosures: **A. Ozkizilcik:** None. **A. Sharma:** None. **D.F. Muresanu:** None. **J.V. Lafuente:** None. **Z.R. Tian:** None. **R. Patnaik:** None. **H. Moessler:** None. **H.S. Sharma:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.10/K3

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

Support: Air Force Material Command, USAF, under grant number FA8655-05-1-3065

Astra Zeneca Mölndal, Sweden

Air Force Office of Scientific Research (EOARD, London, UK),

Swedish Medical Research Council (Nr 2710-HSS),

Swedish Strategic Research Foundation, Stockholm, Sweden

Göran Gustafsson Foundation, Stockholm, Sweden (HSS),

Ministry of Science & Technology, Govt. of India & Govt. of Sweden (HSS/AS)

Title: Nanowired delivery of Bradykinin BK2 receptor antagonist HOE-140 induces neuroprotection in heat stroke through nitric oxide synthase and dynorphin downregulation

Authors: *L. FENG[1], A. SHARMA[2], D. F. MURESANU[3], J. V. LAFUENTE[4], A. OZKIZILCIK[5], Z. R. TIAN[5], H. S. SHARMA[2];
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Abstract: Heat stroke (HS) affects millions of civilians and military personnel Worldwide during summer months when the ambient temperature reaches beyond 38°C. HS induces breakdown of the blood-brain barrier (BBB) in several areas e.g., cerebral cortex, hippocampus, hypothalamus, cerebellum, thalamus and brain stem leading to profound brain edema formation

and neuronal, glial and cellular injuries when exposed at 38°C for 4 h in a biological oxygen demand (BOD) incubator (relative humidity 45-47 %; wind velocity 20-25 cm/sec). Previous reports from our laboratory showed massive upregulation of dynorphin A (1-17) and neuronal nitric oxide synthase (nNOS) in the above brain areas that correlated well with brain pathology. Since bradykinin is potent mediator of brain edema and interacts with NOS, it is possible that this effect of the peptide is mediated through bradykinin receptors. In a model of spinal cord injury (SCI) we have shown that bradykinin B2 receptor antagonist HOE-140 is able to thwart nNOS upregulation and cord pathology. Thus, a possibility exists that HOE-140 may also have neuroprotective effects in HS via suppression of nNOS. Our previous study demonstrated a close interaction between NOS and dynorphin A (1-17) upregulation in SCI. Thus, it appears that in HS both bradykinin and NOS may interact synergistically in HS to induce brain pathology. In present investigation, we examined TiO2 nanodelivery of HOE-140 in HS induced brain pathology and NOS and dynorphin expression in the brain. Our observations showed that HS resulted in significant increase in nNOS and Dynorphin immunoreactivity in almost identical brain areas that showed neuronal, glial and axonal injuries. This indicates that HS is capable to induce both nNOS and Dynorphin A upregulation that is responsible for brain pathology. In separate groups of rats when HOE-140 was administered (1 or 2 mg/kg, s.c.) either 30 min before or 1 h after HS resulted in mild to moderate reduction in nNOS and dynorphin expression. In these rats BBB permeability, brain edema and neuronal injuries were reduced slightly but significantly. However, when TiO2 nanowired HOE-140 was given 60 to 90 min after HS, marked reduction in nNOS and dynorphin immunoreactivity was seen. In these groups of rats BBB breakdown, edema formation and cellular injury were markedly inhibited. These observations are the first to show that blockade of bradykinin B2 receptors effectively using nanodelivery of HOE-140 is capable to induce profound neuroprotection and this effect of well correlated with reduction in nNOS and dynorphin A expression in the neurons, glial cells and endothelial cells, not reported earlier.

Disclosures: **L. Feng:** None. **A. Sharma:** None. **D.F. Muresanu:** None. **J.V. Lafuente:** None. **A. Ozkizilcik:** None. **Z.R. Tian:** None. **H.S. Sharma:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.11/K4

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

Support: Air Force Office of Scientific Research (EOARD, London, UK), and Air Force Material Command, USAF, under grant number FA8655-05-1-3065

Swedish Medical Research Council (Nr 2710-HSS)

Swedish Strategic Research Foundation, Stockholm, Sweden

Title: Endocytosis of Nanomedicines: the case of glycopeptide engineered PLGA nanoparticles

Authors: *G. TOSI[1], B. RUOZI[1], A. VILELLA[3], F. PEDERZOLI[2], F. FORNI[4], M. ZOLI[4], M. A. VANDELLI[4], D. BELLETTI[4], A. SHARMA[5], H. S. SHARMA[5];
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Abstract: The success of nanomedicine as new strategy for drug delivery and targeting prompted the interest to develop approaches toward basic and clinical neuroscience [1]. Despite enormous advances in brain research, central nervous system (CNS) disorders remain the world's leading cause of disability in part due to the inability of the majority of drugs to reach the brain parenchyma. Many attempts to use nanomedicines as CNS drug delivery systems (DDS) were performed; among the various non-invasive approaches, nanoparticulate carriers and particularly polymeric nanoparticles (NPs) seem to be the most interesting strategies [2,3]. In particular, the ability of poly-lactide-co-glycolide NPs (PLGA-NPs) specifically engineered with a glycopeptide (g7), conferring to NPs ability to cross the blood brain barrier (BBB) in rodents at a concentration of up to 10% of the injected dose, was demonstrated in previous studies after different routes of administrations. Most of the evidence on NP uptake mechanisms reported in the literature about intracellular pathways and processes of cell entry is based on *in vitro* studies. Therefore, beside a particular attention devoted to increase the knowledge of the rate of *in vivo* BBB crossing of nanocarriers, the subsequent exocytosis in the brain compartments, their fate and trafficking in the brain surely represent main topics in this field. References 1. Chhabra R, Grabrucker AM, Veratti P, Belletti D, Boeckers TM, Vandelli MA, Forni F, Tosi G, Ruozi B. Characterization of lysosome-destabilizing DOPE/PLGA nanoparticles designed for cytoplasmic drug release. Int J Pharm. 2014 Aug 25;471(1-2):349-57 2. Tosi G, Vilella A, Chhabra R, Schmeisser MJ, Boeckers TM, Ruozi B, Vandelli MA, Forni F, Zoli M, Grabrucker AM. Insight on the fate of CNS-targeted nanoparticles. Part II: Intercellular neuronal cell-to-cell transport. J Control Release. 2014 Mar 10;177:96-107 3. Vilella A, Tosi G, Grabrucker AM, Ruozi B, Belletti D, Vandelli MA, Boeckers TM, Forni F, Zoli M. Insight on the fate of CNS-targeted nanoparticles. Part I: Rab5-dependent cell-specific uptake and distribution. J Control Release. 2014 Jan 28;174:195-201.

Disclosures: **G. Tosi:** None. **B. Ruozi:** None. **A. Vilella:** None. **F. Pederzoli:** None. **F. Forni:** None. **M. Zoli:** None. **M.A. Vandelli:** None. **D. Belletti:** None. **A. Sharma:** None. **H.S. Sharma:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.12/K5

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

Support: Air Force Office of Scientific Research (EOARD, London, UK), and Air Force Material Command, USAF, under grant number FA8655-05-1-3065

India-EU Research Co-operation Program (RP/AS/HSS)

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Swedish Strategic Research Foundation, Stockholm, Sweden

Indian Medical Research Council, New Delhi, India (HSS/AS);

Ministry of Science & Technology, Govt. of India & Govt. of Sweden (HSS/AS)

IT 794/13 (JVL), Government of Basque Country

Title: Nanowired cerebrolysin reduces Sleep deprivation induced regional brain derived neurotrophic factor decline and exacerbation of brain pathology and behavioral dysfunctions

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Abstract: Sleep deprivation (SD) is a serious problem in military as they have only quick nap for few hours under severe stressful conditions leading to profound mental and cognitive dysfunctions. We have previously shown that SD of 12 to 48 h causes blood-brain barrier (BBB) disruption, brain edema formation and neuronal damages. In this investigation we examined a

combination of emotional stress with SD on brain pathology and behavioral dysfunction in a rat model. We also measured regional distribution of brain derived neurotrophic facto (BDNF) in SD. Finally, we used a multimodal drug Cerebrolysin that is a balanced composition of several neurotrophic factors and active peptide fragments to reduce SD induced brain pathology and behavioral dysfunction. Male Wistar rats were subjected to 1 h partial immobilization in plastic tube for 2 weeks to simulate emotional depression. These rats were placed on an inverted flowerpot (7 cm diameter) kept in a pool (30±1°C) for SD where the water level was just below 6 cm from the surface. Control group of rats was placed directly on the flowerpot without any prior stress. In these normal or stressed rats after 48 h SD BBB, brain water content and neuronal damages were examined. Regional BDNF levels were measured using ELISA in cerebral cortex, hippocampus, thalamus, hypothalamus, cerebellum and brain stem. About 70 to 120 % increase in the BBB permeability to Evans blue albumin and radioiodine in the above brain regions was seen in stressed rats after SD as compared to normal rats. The brain water content rose by more than 2 % than in normal animals after identical period of SD. Neuronal injuries were 50 to 90 % more in than normal animals after identical SD. Measurement of BDNF showed 50 to 60 % decline in different brain areas in stressed animals than SD alone. The most marked decrease was seen in hippocampus (normal control 5. 41±0.02 to 2.1±0.08 normal SD; Stress+SD 1.12±0.02 pg/g) and hypothalamus (control 4.23±0.21 to 2.11±0.12 normal SD; Stress+SD 0.84±0.02 pg/g) followed by cortex and cerebellum. Cerebrolysin (2.5 ml/kg, i.v.) given in rats 4 to 6 h after the onset of SD significantly reduced brain pathology and enhanced regional BDNF levels after 48 h SD. However, this effect was not seen in stressed rats after SD. TiO2 nanodelivered cerebrolysin given after the onset of SD in stressed rats significantly enhanced the regional BDNF level and induced neuroprotection at 48 h. Cerebrolsyin treated animals also did not show behavioral dysfunction. These results are the first to show that stress aggravates SD induced behavioral dysfunction and brain pathology and nanodelivery of cerebrolysin has profound neuroprotective effects, not reported earlier.

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Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.13/K6

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

Support: Air Force Office of Scientific Research (EOARD, London, UK), and Air Force Material Command, USAF, under grant number FA8655-05-1-3065

India-EU Research Co-operation Program (RP/AS/HSS)

Swedish Medical Research Council (Nr 2710-HSS)/AS

Swedish Strategic Research Foundation, Stockholm, Sweden

Indian Medical Research Council, New Delhi, India (HSS/AS);

IT 794/13 (JVL), Government of Basque Country

UFI 11/32 (JVL); University of Basque Country, Spain

Title: Repeated forced swim stress exacerbates methamphetamine-induced neurotoxicity. Neuroprotective effects of nanowired delivery of a 5-HT3-receptor antagonist ondansetron

Authors: *J. V. LAFUENTE[1], A. SHARMA[2], E. A. KIYATKIN[3], D. F. MURESANU[4], R. PATNAIK[5], H. S. SHARMA[2];
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Abstract: Stress induced depression is associated with a part of human life. However, sometimes to cope with emotional stress or depression people across the Globe use psychostimulants for temporary pleasure or to get rid of stress induced effects momentarily. However, during this process gradually they are addicted to psychostimulants abuse. One of the most abused drugs is methamphetamine (METH) that leads to often neurotoxicity depending on the dose and duration of drug exposure. Furthermore, METH induced effects could be aggravated at both hot and cold environment. One of the key reasons for METH induced neurotoxicity is the breakdown of the blood-brain barrier (BBB) leading to development of edema and cellular injuries. Since strenuous stress by itself leads to BBB breakdown and cellular injuries, a possibility exists that combinations of stress and METH use could exacerbate brain damage. Forced swim (FS) stress and concomitant immobility is considered to be a model of depression. Pretreatment with drugs modifying serotonin metabolism reduces depressive episodes indication a role of serotonin in depressive illness. Administration of METH also known to alter serotonin metabolism. Thus, it would be interesting to find out whether repealed forced swim and concomitant depressive episodes could aggravate METH induced neurotoxicity in a rat model. Male Wistar rats (age 20 to 24 weeks) were subjected to FS in a pool (30°C) for 15 min daily for 8 days. Control rats kept at room temperature (21±1°C) for comparison. In these control and FS rats METH was administered (9 mg/kg, s.c.) and allowed to survive 4 h after drug

injection. METH treatment in FS rats induced a profound increase in the BBB breakdown to Evans blue albumin (EBA) by 150 to 220 % and [131]-Iodine by 250 to 380 % from the naive rats received identical dose of METH. The BBB leakage was most pronounced in the cerebrla cortex followed by hippocampus, cerebellum, thalamus and hypothalamus in both FS and naïve rats after METH intoxication. Development of brain edema was also 2 to 4 % higher in FS rats after METH than in naive animals. Neuronal and glial cell injuries were also aggravated by 3- to 5-fold in FS rats after METH. Pretreatment with ondansetron (1 mg/kg, i.p.) 30 min before METH injection in naïve rats reduced the brain pathology. However, TiO2-nanowired delivery of ondansetron (1 mg/kg, I,p,) was needed to reduce brain damage, BBB leakage and edema formation in FS rats after METH administration. Taken together these results are the first to show that METH exacerbates neurotoxicity in depressive animals and this effects is mediated though 5-HT3 receptors, not reported earlier.

Disclosures: **J.V. Lafuente:** None. **A. Sharma:** None. **E.A. Kiyatkin:** None. **D.F. Muresanu:** None. **R. Patnaik:** None. **H.S. Sharma:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.14/K7

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

Support: Air Force Office of Scientific Research (EOARD, London, UK), and Air Force Material Command, USAF, under grant number FA8655-05-1-3065

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Swedish Strategic Research Foundation, Stockholm, Sweden

Indian Medical Research Council, New Delhi, India (HSS/AS);

Alexander von Humboldt Foundation, Germany (HSS/AS)

IT 794/13 (JVL), Government of Basque Country

Title: SiO2-nanoparticles associated with hypertension exacerbates blood-brain barrier breakdown, edema formation and cellular injuries following traumatic brain injury

Authors: *A. NOZARI[1], A. SHARMA[2], J. V. LAFUENTE[3], D. F. MURESANU[4], R. PATNAIK[5], H. MOESSLER[6], H. S. SHARMA[2];
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Abstract: Military personnel during combat operation are often exposed to long-term chronic stress leading to mild to moderate work-related hypertension. In addition, in desert areas additional exposure of silica dust (SiO2 nanoparticles, SiO2NPs) could make their situation even worse. Under such circumstances, if combat related trauma to the central nervous system (CNS) will occur, the pathophysiological outcome may be more worse than similar trauma occurring in the absence of SiO2 NPs and/or hypertension. Thus, there is an urgent need to explore the possibility of hypertension and SiO2NPs exposure modulating the pathophysiology of traumatic brain injuries (TBI) in model experiments to find out suitable therapeutic strategies. Male Wistar rats (250-300 g) were made renal hypertensive by 2kidney 1clip (2K1C) procedure allowing mean arterial blood pressure (MABP) reaching 180±8 torr over 6 weeks. These hypertensive rats were exposed to SiO2NPs (40-50 nm) once daily (50 mg/kg, i.p.) for 8 days. On the 9th day these hypertensive and SiO2NPs intoxicated animals were subjected to TBI under anesthesia by making an incision (3 mm long and 2.5 mm deep) on the right parietal cerebral cortex after opening the skull (4mm OD) on both sides. The animas were allowed to survive 24 and 48 h after TBI. A focal TBI in hypertensive and SiO2NPs rats resulted a progressive increase in BBB breakdown to Evans blue albumin (EBA) and [131]-Iodine by 4-to 6- fold from the normal saline treated rats after 24 and 48 h TBI respectively. These animals also showed pronounced exacerbation of edema development in the injured hemisphere by 4- to 8 % and in the uninjured hemisphere by 2- to 3 % at 48 h TBI as compared to saline treated healthy animals. Marked neuronal, glial and myelin damage is seen in hypertensive rats with SiO2NPs exposure after TBI as compared to saline treated normal rats. The behavioral dysfunctions as seen using Rota-Rod apparatus, walking on an inclined mesh-grid and gait analysis also showed marked exacerbation in these hypertensive rats with SiO2NPs exposure at 48 h TBI. Treatment with a multimodal drug Cerebrolysin-a balanced composition of several neurotrophic factors and active peptide fragments in a dose of 10 ml/kg, i.v. started after 8 h TBI and continued every 8 h intervals (5 injections) markedly reduced brain pathologies in hypertensive and SiO2 intoxicated rats. Whereas only 5 ml/kg of the drug is needed to achieve identical neuroprotection in saline treated normal rats after TBI. These observations are the first to show that a combination of hypertension and SiO2NPs worsens brain pathology in TBI and require double dose of drugs to induce neuroprotection, not reported earlier.

Disclosures: A. Nozari: None. **A. Sharma:** None. **J.V. Lafuente:** None. **D.F. Muresanu:** None. **R. Patnaik:** None. **H. Moessler:** None. **H.S. Sharma:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.15/K8

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

Support: Air Force Material Command, USAF, under grant number FA8655-05-1-3065

India-EU Research Co-operation Program (RP/AS/HSS)

Swedish Medical Research Council (Nr 2710-HSS)

Swedish Strategic Research Foundation, Stockholm, Sweden

Astra Zeneca, Mölndal, Sweden (HSS/AS)

IT 794/13 (JVL), Government of Basque Country and UFI 11/32 (JVL) University of Basque Country, Spain

UFI 11/32 (JVL); University of Basque Country, Spain.

Title: Nanodelivery of Mesenchymal Stem Cells with Cerebrolysin potentiates neprilysin level and decreases brain pathology and amyloid-beta peptide in Alzheimer's disease

Authors: *H. S. SHARMA[1], D. F. MURESANU[2], J. V. LAFUENTE[3], Z. TIAN[4], A. OZKIZILCIK[4], H. MOESSLER[5], R. PATNAIK[6], A. SHARMA[7];
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Abstract: Neprilysin (NPL) is an endogenous enzyme that functions as rate-limiting step in amyloid-beta peptide (AβP) degradation. It is widely believed that an imbalance between production and clearance of AβP results in its accumulation leading to development of Alzheimer's Disease (AD). In several cases of AD the metalloprotease NPL brain concentration is decreased. Also NPL knocked out mice exhibited AD like brain pathology and behavioural dysfunctions. This suggests that enhancing the NPL concentartions by therapeutic means may reduce brain pathology in AD. Few studies indicated that mesenchymal stem cells (MSCs) administration into the brain fluid compartment resulted in enhanced NPL concentration and a

reduced AβP accumulation that correlates well with the behavioural and pathological deficit in a mouse model of AD. Thus, it would be interesting to examine whether nanodelivery of MSCs may further potentiate NPL enhancement and reduction in the AβP levels in the brain in an animal model of AD. Our previous studies have shown that TiO2-nanowired cerebrolysin (CBL), a balanced composition of several neurotrophic factors and active peptide fragments is able to thwart AD pathology following AβP infusion model in rats. However, role of CBL on NPL concentration are not well known. Thus, in present investigation we explored the combined effects of of nanowired MSCs and CBL on NPL and AβP levels in our rat model of AD. AD like symptoms was produced in rats by administering AβP (1-40) intraventricularly (i.c.v.) in the left lateral ventricle in a dose of 250 ng/10 µl once daily for 4 weeks. After 30 days of the 1st AβP infusion, the rats were examined for NPL and AβP concentrations in their brain by ELISA and brain pathology using histopathological techniques. Our observations showed that co-administration of TiO2 nanowired MSCs (106 cells) with 2.5 ml/kg CBL (i.v.) once daily for 1 week starting from 2 weeks after AβP infusion resulted in a significant increase of NPL in hippocampus (400 pg/g) from AβP group (120 pg/g; Control 420±8 pg/g brain). Also the combined treatment resulted in significant decrease of hippocampal AβP in treated group (45 pg/g) as compared to AβP group alone (75 pg/g; control 40±4 pg/g). Interestingly these changes were 30 to 45 % less by MSCs or CBL treated alone. The brain pathology in terms of neuronal damage, gliosis and myelin vesiculation was also most protected in combined treatment with TiO2 MSCs and CBL in our AD model. These observations are the first to show that a combination of CBL and MSCs has superior neuroprotective effects in AD by increasing the NPL level effectively, not reported earlier.

Disclosures: **H.S. Sharma:** None. **D.F. Muresanu:** None. **J.V. Lafuente:** None. **Z. Tian:** None. **A. Ozkizilcik:** None. **H. Moessler:** None. **R. Patnaik:** None. **A. Sharma:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

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Program#/Poster#: 689.16/K9

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

Support: CONACYT 288760/233854

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CONACYT 129303

Title: Cerebrolysin improves memory and learning of the spontaneously hypertensive rats

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Abstract: Hypertension (HT) is a disease characterized by a sustained increase in blood pressure. This condition is a major risk factor for suffering cerebrovascular disease, such as ischemic or hemorrhagic stroke, microvascular injury, cognitive impairment and vascular dementia. Experimentally, the model of spontaneously hypertensive (SH) rats has been widely used to study alterations at the anatomical, physiological and behavioral level, which develop by the progression of HT. The SH animals shown an increase in the blood pressure after second month affecting brain areas such as hippocampus and prefrontal cortex. The pyramidal neurons of these areas exhibited retraction of the dendritic arborization and reduced number of the dendritic spines. In the present report we analyzed the effect of chronic administration (6 month) of the Cerebrolysin (CBL), a polypeptide preparation with neurotrophic effects similar to those of endogenous neurotrophic factors, on the memory and learning and neural morphology of the pyramidal neurons of CA1 and dentate gyrus (DG) of the dorsal hippocampus and, layers III and V of prefrontal cortex by using Golgi-Cox stain. Wistar Kyoto rats were used as control animal groups and all animals used in this study were female rats. CBL improved learning and memory in the SH rats but not in WKY, measured by Water-Morris test. In addition, pyramidal neurons of layers III and V of the PFC and, CA1 and DG of dorsal hippocampus of the SH rats which received CBL, shown more arborization with increased number of dendritic spines. WKY rats shown more number of dendritic spines only in neurons of the DG of the hippocampus. Our results suggest that CBL is a potential drug in the treatment of the cognitive impairment caused by HT.

Disclosures: **V. Cabrera Pedraza:** None. **C. Solis:** None. **R. Vazquez-Roque:** None. **F. De la Cruz:** None. **S. Zamudio:** None. **M. Gomez-Villalobos:** None. **G. Flores:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.17/K10

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

Support: Swiss National Science Foundation

Title: L-Lactate as a neuroprotective agent against excitotoxicity: implication of an energy-dependent process

Authors: *P. JOURDAIN[1,2], I. ALLAMAN[1], P. MARQUET[2], P. J. MAGISTRETTI[3,1,2]; [1]EPFL, Lausanne, Switzerland; [2]CNP-CHUV, Prilly, Switzerland; [3]KAUST, Thuwal, Saudi Arabia

Abstract: There are several reports indicating a neuro-protective action of L-Lactate (Ros et al., 2001; Berthet et al., 2009, 2012). We have recently undertaken an analysis of this neuro-protective effect by using Digital Holographic Microscopy (DHM), a new imaging technique able to detect early signs of cell death in culture (Jourdain et al., 2011). Neuronal death elicited by application of glutamate (100μM; 2min) was detected in 65% of studied cells. This process is mainly linked to the activation of NMDA receptors since it was blocked by APV and MK801. In the presence of L-Lactate, the percentage of neuronal death induced by glutamate was significantly decreased to 32%. The neuro-protective action of L-Lactate was mimicked by L-pyruvate (but not by D-Lactate). UK5099, a specific blocker of mitochondrial pyruvate carrier, fully prevented the L-lactate-mediated neuroprotection, suggesting that the neuro-protective effects induced by L-Lactate required the synthesis of ATP. In addition, L-Lactate was not able to neuroprotect in presence of specific blockers of ATP channels pannexins (probenicid and carbenoxolone), nor in the presence of apyrase, an enzyme degrading ATP. However, ATPγS (10μM) in the extracellular medium was sufficient to rescue a neuroprotective effect even in presence of apyrase. These results indicate that ATP (and not its metabolites like adenosine) produced by the L-Lactate/Pyruvate pathway could be released to act on purinergic receptors. Using specific agonists and antagonists, we demonstrated that P2Y2 was the main purinergic receptor involved in neuroprotection. Finally, LY294002, a specific blocker of the PI3K pathway, blocked also the neuro-protective effect of L-Lactate. Interestingly, this intracellular pathway is activated by P2Y2. Altogether these data demonstrate that L-lactate confers neuroprotection from excitotoxic insults through complex intracellular pathways relying on an increase neuronal energy substrates availability, a release of ATP acting on purinergic receptors, which results in the mobilization of the PI3K intracellular pathway.

Disclosures: **P. Jourdain:** None. **I. Allaman:** None. **P. Marquet:** None. **P.J. Magistretti:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

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Program#/Poster#: 689.18/K11

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

Support: Hutton Honors College Grant

Title: Neuroprotection with androgens following partial motoneuron depletion: A role for microglia

Authors: B. J. KILEY, *D. R. SENGELAUB;
Indiana Univ., Bloomington, IN

Abstract: Neurodegenerative disease or nerve injury results in the loss of spinal motoneurons, and remaining motoneurons show a variety of morphological and functional changes. We have previously demonstrated that partial depletion of motoneurons innervating the quadriceps muscles induces dendritic atrophy in remaining motoneurons, with 70% decreases in dendritic length. Treatment with testosterone is neuroprotective, and dendritic atrophy following partial motoneuron depletion is attenuated. In the present study, we explored a potential mechanism for this induced atrophy and the protection by androgen treatment, examining the response of microglia to the partial depletion of motoneurons with and without testosterone treatment. Microglia are activated locally and recruited from other sites in response to injury. Microglia are involved in the removal of synapses and dendrites after injury, and there is evidence that their activation is influenced by steroid hormones. Motoneurons innervating the vastus medialis muscle in adult male rats were selectively killed by intramuscular injection of cholera toxin-conjugated saporin. Simultaneously, saporin-injected rats were given systemic treatments via interscapular implants containing testosterone or left blank. One or three weeks later, microglia were visualized after immunohistochemical staining for Iba1. Microglia surrounding the injured motoneurons were classified as monitoring or activated (primed, reactive, or ameboid) based on morphology and counted stereologically. Compared with intact males, partial motoneuron depletion resulted in increases in the total number of microglia (78% and 24% at 1 and 3 weeks post-saporin, respectively) in the quadriceps motor pool. These changes were driven by increases in the number of activated microglia compared to levels found in intact animals; the number of activated microglia increased by 144% at 1 week post-saporin, and remained elevated at 3 weeks (51%). The increases in the number of activated microglia were attenuated with testosterone treatment; the number of activated forms increased only 34% and 17% at 1 and 3 weeks post-saporin, respectively. These findings suggest that the dendritic atrophy observed in remaining motoneurons after partial motoneuron depletion could be a result of increased microglial activation in the injury site, resulting in collateral damage through synaptic stripping and dendritic loss. The attenuation of both dendritic atrophy and microglial activation with testosterone treatment supports this potential causal effect, and further supports a role for hormones as neurotherapeutic agents in the injured nervous system.

Disclosures: **B.J. Kiley:** None. **D.R. Sengelaub:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.19/K12

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

Support: Conacyt Grant 127357

Title: AhR null mice are protected against neurotoxic insult

Authors: *L. G. GARCIA[1], F. PEREZ-SEVERIANO[2], D. GONZALEZ-ESQUIVEL[2], G. ELIZONDO[1], J. SEGOVIA-VILA[1];
[1]CINVESTAV, D.F., Mexico, Mexico; [2]Inst. Nacional de Neurología y Neurocirugía, Mexico City, Mexico

Abstract: L-kynurenine (Kyn) is a key element of the tryptophan metabolism; it is enzymatically converted by kynurenine aminotransferase II (KAT II) to kynurenic acid (KYNA), which acts as an antagonist to the NMDA receptor-glycine site. Kyn is also an endogenous ligand of the aryl hydrocarbon receptor (AhR), a transcription factor that regulates the expression of a diverse set of genes. Interestingly, KYNA levels are reduced in several regions of the brain of Huntington's disease (HD) patients. In the present work we used an AhR-null mouse and age-matched wild type mice to determine the effect of the absence of AhR on KYNA bioavailability. Moreover, we induced an excitotoxic insult by the intrastriatal administration of quinolinic acid, which is a biochemical model of HD, in both AhR-null and wild type mice in order to evaluate the neurological damage as well as the oxidative stress caused by the lesion. We found that in AhR-null mice there is an increase of KYNA levels in specific brain areas which are associated with higher expression of KAT II and this induces a neuroprotective effect against the neurotoxic insult. We also observed a decreased expression of the NR1 subunit of the NMDA glutamate receptor, which could be related to a decreased excitatory neurotransmission. Additionally, AhR-null mice also show improved motor performance in the rotarod test, indicating a constitutive protection of striatal tissue.

Disclosures: L.G. Garcia: None. **F. Perez-Severiano:** None. **D. Gonzalez-Esquivel:** None. **G. Elizondo:** None. **J. Segovia-Vila:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

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Program#/Poster#: 689.20/K13

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

Support: Linköping University

Region Östergötland

Title: The effects of low and high doses of 17β-estradiol on focal cerebral ischemia in rats

Authors: *E. INGBERG, E. THEODORSSON, A. THEODORSSON, J. O. STRÖM; Linköping University/Ike, Linköping, Sweden

Abstract: Although the majority of the studies of the effects of estrogens on focal cerebral ischemia in rodents have shown neuroprotection, there are also some contradicting studies in which increased damage have been observed. Differences in hormone administration methods, resulting in different serum 17β-estradiol concentrations has been suggested to account for this dichotomy. To test this hypothesis, female wistar rats were ovariectomized and randomized into three groups differing in administered dose of 17β-estradiol and subsequently subjected to transient middle cerebral artery occlusion using the filament method. After 24 h, sensorimotor function was evaluated with the tape test before the animals were sacrificed for infarct size measurement. No differences were found between the groups regarding either infarct size or behavioral performance and hence no support was found for the hypothesis of administered dose as the crucial factor determining the effects of 17β-estradiol.

Disclosures: E. Ingberg: None. **E. Theodorsson:** None. **A. Theodorsson:** None. **J.O. Ström:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

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Program#/Poster#: 689.21/K14

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

Support: TCVGH-HK1048009

Title: The effects of hemoxygenase-1 on lipopolysaccharide-induced neuroinflammation in astrocyte cultures

Authors: *J.-Y. WANG[1], C.-L. CHEN[2], S.-Y. CHEN[3];
[1]Dept Nursing (Basic Med. Sci), Hungkuang Univ., Taichung, Taiwan; [2]Li-Shin Hosp., Taoyuan, Taiwan; [3]Dept. of Nursing (Basic Med. Science), Taichung, Taiwan

Abstract: Heme oxygenase-1, a stress protein, may play a cytoprotective role in various neuronal injury. The induction of HO-1 is a sensitive marker of tissue oxidative stress. Furthermore, the changes in brain HO-1 expression patterns were associated with aging processes. In AD temporal cortex and hippocampus revealed more intense HO-1 band than in normal. *In vivo* and *in vitro* studies indicated that enhanced HO-1 activity may either ameliorate or exacerbate neuronal injury. Brain-derived neurotrophic factor (BDNF) exerts a neuroprotective effect against ischemic brain injury. Evidence revealed that the neuroprotective mechanisms of HO-1 may be related to the activation of the BDNF/TrkB/PI3K/Akt signaling pathway. However, the role of HO-1 in neuronal injury is still not clear. Alzheimer's disease (AD) is the most common neurodegenerative disorder. It is associated with relevant neuroinflammatory response. Astrocytes, a kind of glial cell, can produce mediators that involve in neuroinflammation. The processes accompany with inflammatory response are increasing in the expression of transcription factor NFκB, inducible nitric oxide synthase (iNOS) and the release of inflammatory mediators (ex. nitric oxide (NO) and cytokines). Ultimately, these events may create a vicious cycle to induce neuronal injury. In this study we want to estimate the effects of hemoxygenase-1 (HO-1) and BDNF on LPS-induced inflammation and oxidative stress in astrocytes (SVGp12). Cells cultures will be subjected to (1) control; (2) LPS treated with 1, 10, 100, 1000 ng/ml; (3) CoPP (HO-1 inducer) treated alone with 1, 2, 4, 10 μM; (4) pretreatment of CoPP following LPS treatment for 1, 3 or 5 days. Cell injury will be assessed by 3-(4, 5-Dimethyl thianol-2-yl) 2, 5 di-phenyltetrazolium bromide (MTT) reduction. The activation of astrocytes will visualize using anti-GFAP by immunocytochemistry. The production of MDA and NO will be measured to estimate the oxidative stress and inflammation. The expression of HO-1 and BDNF protein will be quantified by western blot analysis. Our data indicated that the NO production was increasing and the MTT reduction was decreasing in primary rat mixed glial cultures exposure to LPS. However, there was no significant difference in same condition of astrocyte cultures. The expression of HO-1 and BDNF protein were no significant change, too. BSO (will deplete glutathione in cells) 1000 μM induced cell injury, the percentage of MTT reduction decreasing to below 50 %. About the effects of HO-1 on neuroinflammation, we need more investigation.

Disclosures: J. Wang: None. **C. Chen:** None. **S. Chen:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

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Program#/Poster#: 689.22/K15

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

Support: NSERC Canada 6721

Title: Differential targeting of HSPA (HSP70) heat shock protein family members in human neuronal cells following cellular stress

Authors: C. A. DEANE, *I. R. BROWN;
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Abstract: HSPA6 (Hsp70B`) is a little studied member of the HSPA (HSP70) family of heat shock proteins that is present in the human genome but not rat and mouse and is therefore missing in current animal models of neurodegenerative diseases. HSPA6 shares 84% sequence homology with the more widely studied HSPA family member, HSPA1A (Hsp70-1). It is not known whether HSPA6 exhibits similar or divergent functions compared to HSPA1A. Hsps are highly conserved proteins that play roles in cellular repair and protective mechanisms. Co-application of celastrol and arimoclomol to differentiated human neuronal SH-SY5Y cells at dosages that do not affect viability or neuronal process morphology results in synergistic induction of a set of Hsps, including HSPA6. The induced proteins are diffusely distributed in the neuronal cytoplasm. Subsequent application of thermal stress triggers a transient, mass movement of HSPA6 and HSPA1A into the nucleus and targeting to discrete nuclear substructures. HSPA1A localizes to nuclear speckles rich in RNA splicing factors and to the nucleolus that is involved in ribosome biogenesis. HSPA6 translocates to the periphery of nuclear speckles that are sites of RNA transcription during recovery from thermal stress. The observed differences in nuclear targeting of endogenously induced HSPA6 compared to HSPA1A suggest divergent functions of these components of the cellular stress response.

Disclosures: C.A. Deane: None. **I.R. Brown:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

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Program#/Poster#: 689.23/K16

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

Support: DTRA

Title: Inhibition of the IL-1 and TNF signaling pathways for neuroprotection is additive following acute soman-induced seizure in mice

Authors: *E. A. JOHNSON, J. IRWIN, K. LAITIPAYA, J. CHANDLER, L. SHUMWAY, T. FERRARA-BOWENS, M. WEGNER;
Pharmacol., US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: Exposure to organophosphorus compounds such as soman (GD) can initiate status epilepticus (SE) that can lead to progressive brain damage and behavioral impairment. GD, a potent acetylcholinesterase inhibitor and chemical warfare nerve agent, causes prolonged SE activity and cell death in the hippocampus, thalamus and piriform cortex. Current treatments can ameliorate GD-induced SE, though treatment effectiveness diminishes rapidly following exposure. Therefore, neuroprotective strategies that can be used at later time points are needed to reduce neurodegeneration and improve cognitive outcomes. One strategy involves modulation of the neuroinflammatory response, a prominent feature in GD-induced brain injury. Previous studies have shown that both the tumor necrosis factor (TNF) and interleukin (IL)-1 signaling systems play a role in neuroprotection though the brain regions protected are specific to each pathway. This study focused on whether inhibition of both signaling pathways was a viable neuroprotective strategy that could confer greater neuroprotection in more regions than either pathway alone. A background-matched IL-1 receptor 1 (IL-1R1)/TNF receptor 1A (TNFR1A) double knockout (KO) mouse strain was exposed to GD and showed significant improvements in neuropathology, mortality, seizure onset and other relevant physiological responses compared to wild type, IL-1R1 KO and TNFR1A KO mice. These results suggest that a multi-pathway approach may be necessary to greatly improve brain injury outcomes following seizurogenic exposure to CWNA. Also, the use of multiple KO strains can help guide the selection of pathway-specific drugs for neuroprotection testing. These studies describe a rational and relatively rapid therapeutic strategy for the development of effective neuroprotectants and show that neuroprotection is possible with a longer therapeutic window after GD exposure than is provided by conventional treatments.

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Poster

689. Neurotoxicity: Protective Mechanisms

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

Support: NIH Grant AG027956

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Felix and Carmen Sabates Missouri Endowed Chair in Vision Research

Vision Research Foundation of Kansas City

Title: Control of neuronal ryanodine receptor mediated calcium signaling by steroid hormone receptors

Authors: *P. KOULEN;
Basic Med. Sci. & Ophthalmology, Univ. of MO - Kansas City, Kansas City, MO

Abstract: Controlling the cytoplasmic concentration of free calcium ions (Ca^{2+}) is essential for the physiological activity of neurons and for neuronal survival under disease conditions. At the same time, the steroid hormones progesterone and the estrogen 17β-estradiol have been identified as neuroprotective and critical for proper neuronal viability. The present study determined novel mechanisms of action how signaling mediated by the interaction of ryanodine receptors with steroid hormone receptors controls the intracellular Ca^{2+} concentration in neurons. Specifically, it was identified how direct binding of cytoplasmic estrogen receptors to ryanodine receptors affects the activity of this major type of ligand-gated intracellular Ca^{2+} release channels in neurons. Using immunochemistry, optical imaging and electrophysiology, as well as immunochemical assays for determining protein-protein binding, changes in the activity of ryanodine receptors after binding of steroid hormone receptors were determined. Binding of steroid hormone receptors to the intracellular Ca^{2+} release channel resulted in distinct changes in both channel open frequency as well as the number of channel openings at the single channel level and preservation of key biophysical parameters of the channels such as single channel conductance. These molecular changes in channel open probability were mirrored at the cellular level by altered release of Ca^{2+} from intracellular stores and altered susceptibility of neurons to disease stimuli. The work indicates that neuronal Ca^{2+} signaling mediated by ryanodine receptors as Ca^{2+} dependent intracellular Ca^{2+} release channels is critically modulated by steroid hormone receptor binding. Such signaling controlled by protein-protein interactions in the central nervous system potentially provides a novel mechanism for drug development in the area of neurodegeneration.

Disclosures: P. Koulen: None.

Poster

689. Neurotoxicity: Protective Mechanisms

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

Support: Conacyt 152613

PAPIIT IN211913-3

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Title: Glycyrrhizin suppresses oxidative stress in the hippocampus and olfactory bulb on status epilepticus in a lithium-pilocarpine seizure model

Authors: *S. GONZÁLEZ-REYES[1], J. J. SANTILLÁN-CIGALES[2], J. PEDRAZA-CHAVERRI[3], R. GUEVARA-GUZMÁN[2];
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Abstract: Glycyrrhizin (GL), a triterpene, is considered the primary active compound present in the roots and rhizomes of licorice (Glycyrrhiza glabra). Extensive studies documented that GL has diverse biological activities as anti-inflammatory, antiviral and antioxidant, among others. This work was designed to determinate the potential protective effect of GL in the hippocampus and olfactory bulb on status epilepticus in a rat model. Male Wistar rats (220-250 g) were kept under controlled environmental conditions with free access to standard laboratory chow and tap water. Rats were randomly divided into four groups: 1) control of vehicle (CV); 2) status epilepticus (SE); 3) GL before SE (GL+SE); and, 4) only GL. Rats in groups 2 and 3 were injected with lithium chloride (3 meq/kg) 18 hours before pilocarpine (30 mg/Kg i.p.). Rats in group 3 were treated with GL (50 mg/kg i.p.) 30 minutes before pilocarpine injection. To minimize the peripheral cholinergic effects of pilocarpine, scopolamine methyl nitrate (1 mg/kg v.sc) was injected 30 min prior. Tissues were obtained 3 or 24 h after SE was interrupted with diazepam (2 mg/kg i.m). Oxidative stress (OS) was evaluated with glutathione (GSH) levels, malondialdehyde (MDA) content and the enzymatic activities of glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) and superoxide dismutase (SOD). The results showed increases of MDA content after the time of SE in hippocampus and olfactory bulb. GL was able to avoid OS, significantly in both tissues. The content of reduced glutathione (GSH) was maintained with the pretreatment of GL since 3 until 24 h. Also, MDA content was diminished with GL prior SE. Phase II enzymes were decreased with the injury. However, pre-treatment with GL prevented depletion of activities. This work suggested that GL

has a protective role against oxidative stress after SE in hippocampus and olfactory bulb of rats, on early hours (3 and 24h). GL prevents loss of activities of enzymes involved in GSH redox cycle and also in GSH levels and MDA content.

Disclosures: **S. González-Reyes:** None. **J.J. Santillán-Cigales:** None. **J. Pedraza-Chaverri:** None. **R. Guevara-Guzmán:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

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Program#/Poster#: 689.26/K19

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

Title: X-irradiation-induced decrease in drebrin clusters within dendritic spines of cultured hippocampal neurons: Association with NMDA receptor and histone deacetylase activities

Authors: S. MIAO, N. KOGANEZAWA, T. HIRUMA, K. HANAMURA, A. PUSPITASARI, R. T. ROPPONGI, *T. SHIRAO;
Gunma Univ. Grad Sch. Med., Maebashi, Gunma, Japan

Abstract: Therapeutic X-irradiation of the brain possibly causes cognitive impairment, which is associated with synaptic dysfunction. We have shown that the acute effects of X-irradiation on contextual fear memory of adult mice and the immunostaining intensity of a postsynaptic F-actin-binding protein, drebrin in molecular layer of dentate gyrus *in vivo*. Although the decrease in drebrin-immunostaining intensity seems to be associated with fear memory, the mechanism regulating this decrease is unknown. We have shown that glutamate-induced decrease in drebrin clusters within dendritic spines of cultured neurons is mediated by NMDA receptor activity. To examine whether NMDA receptor activity is associated with X-irradiation-induced decrease in drebrin-immunostaining intensity in postsynaptic sites, we used primary hippocampal neuronal culture and analyzed the effect of X-irradiation on drebrin accumulation at dendritic spines *in vitro*. The neurons were treated with 50 μM Amino-5-phosphonovaleric acid (APV; an NMDA receptor antagonist) 1 hour before 10 Gy of X-irradiation at 21 days *in vitro*. The neurons were fixed 8 hours after X-irradiation. Immunocytochemical analysis showed that drebrin cluster density along dendrites significantly decreased 8 hours after X-irradiation ($p < 0.05$). This decrease was blocked by pretreatment with APV, suggesting that NMDA receptor activity is involved in X-irradiation-induced decrease in drebrin clusters within dendritic spines. We have also shown that amyloid beta oligomers-induced decrease in drebrin clusters within dendritic spines of cultured hippocampal neurons is mediated by histone deacetylase (HDAC). Moreover,

it is known that some HDAC inhibitors protect normal cells from radiation-induced damage. Then we are examining the effects of suberoylanilide hydroxamic acid (SAHA, a histone deacetylase inhibitor) on X-irradiation-induced decrease in drebrin clusters. These data suggest that NMDA receptor antagonists and histone deacetylase inhibitors may provide a new avenue toward therapeutic tools to mitigate X-irradiation-induced synaptic dysfunction.

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Poster

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

Support: NIH/NIAAA R03AA022479

Title: Protective effects of PACAP against alcohol-induced toxicity in SH-SY5Y cells

Authors: ***S. MANAVALAN**[1], D. BROWN[2], L. AKINFIRESOYE[2], S. TADESSE[2], K. MANAYE[3], A. TAMAS[1], D. REGLODI[1], Y. TIZABI[2];
[1]Univ. of Pecs, Hungary, Pecs, Hungary; [2]Pharmacol., [3]Physiol., Howard Univ. Col. of Med., Washington, DC

Abstract: Pituitary adenylate cyclase-activating polypeptide (PACAP) is an endogenous 38 amino acid containing neuropeptide with various cytoprotective functions including neuroprotection. Thus, in-vitro and in-vivo studies have provided substantial evidence of PACAP protection against neuronal injury related to ischemia, trauma and various endogenous and exogenous toxic agents. We have earlier reported that exposure of neuroblastoma-derived SH-SY5Y cells to salsolinol, an endogenous toxicant formed through condensation of dopamine and acetaldehyde results in significant cell loss and that pre-exposure to PACAP can protect against this toxicity. In this study, we sought to determine whether PACAP might also protect against alcohol-induced toxicity in these cells and elucidate possible underlying mechanism(s). Ethanol treatment of SH-SY5Y cells resulted in a concentration-dependent toxicity where maximal effect (approximately 42% cell loss) was achieved with 500 mM ethanol. Pretreatment with PACAP resulted in a dose-dependent reduction in ethanol-induced toxicity where at 200 nM, PACAP completely blocked ethanol's maximal effect. The effects of PACAP in turn, were inhibited by PACAP antagonist (PACAP 6-38) where full block was achieved with 2 uM

antagonist. Cell flow cytometry indicated that the major toxicity by ethanol was apoptotically mediated and that PACAP could block this process. Taken together, these data indicate that PACAP or PAC1 receptor agonist, a major site of PACAP action, could be of therapeutic potential in alcohol-induced neurotoxicity. Supported by: NIH/NIAAA R03AA022479 (YT), MTA Momentum Program, Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-III/5., Arimura Foundation and OTKA K104984 (AT, DR), NIA/NIH 1R25AG047843-01 (KM)

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Poster

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

Support: NIH Grant NS076448

Title: Intranasal CNS delivery of catalytic bio-scavengers to protect against organophosphate threat agents: Preliminary studies

Authors: *A. P. APPU, A. PEETHAMBARAN, J. K. S. KRISHNAN, J. R. MOFFETT, A. M. A. NAMBOODIRI;
C-2069, Anatomy, Physiol. and Genet., Uniformed Services Univ. of Hlth. Sci., Bethesda, MD

Abstract: Organophosphate poisoning is a complex international health issue encompassing risks as diverse as insecticide poisoning and chemical threat agent (CTA) exposure. An emerging countermeasure approach is the use of catalytic bioscavengers such as organophosphate hydrolase (OPH) to detoxify the CTAs enzymatically. In recent studies, recombinant variants of OPH and paraoxonase 1 were found to afford protection against paraoxon intoxication in animal models. However, delivery of the active enzyme to the brain to prevent central nervous system (CNS) toxicity remains a challenge due to the blood brain barrier (BBB). We have been developing a method of nose-to-brain delivery for enzymes that bypasses the BBB to deliver these CTA treatments to the CNS. In the present study, we have demonstrated quick delivery of a model enzyme- chloramphenicol acetyltransferase (CAT) alone as well as in combination with matrix metaloproteinase-9 (MMP-9) into different areas of brain using an intranasal brain delivery system (ImpelNeurophrma, Seattle, WA). The enzyme activity in the different brain region was determined using a specific radiometric assay. The results showed that active enzyme

reached all region of the brain within 15 min, with approximately 15-31% of the total enzyme activity reaching the brain under this condition. Based on the results, it appears that both olfactory and trigeminal pathways are involved in the transport of CAT into the brain. Intranasal delivery of enzymes in combination with MMP-9 is a novel strategy to counter CTA toxicity in the CNS. The aim of this study was to provide an effective model for the rapid delivery of active enzymes into the brain in order to treat CTA exposure and various neurological disorders. Our immediate goals are to optimize the current intranasal brain delivery method for OPH in a model system of paraoxon toxicity in the rat with the enzyme delivered alone and in combination with oximes.

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Poster

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

Support: NIH Grant NICHD RO1HD069238

Title: Developmental regulation of stress-inducible neuroprotective protein pDING in human fetal brain with maternal alcohol and SSRI exposure

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Abstract: Introduction: Maternal alcohol exposure can lead to significant neuronal loss, synaptic dysfunction and fetal alcohol syndrome (FAS). We have reported a neuroprotective role for stress-inducible DING phosphatase (pDING) in animal models and in-vitro human models. We hypothesized that pDING has a key protective role in normal human fetal neurodevelopment and that this protective effect may be disrupted in pregnancies exposed to EtOH and/or selective serotonin reuptake inhibitors (SSRIs). Methods: Fetal brain and placental tissue was collected with IRB approval following elective pregnancy termination between 8 and 20 weeks' gestation.

Placental tissue was also collected following term delivery >37 weeks Exposure was classified based on detailed SSRI/EtOH questionnaires. Endogenous pDING levels were assessed in total brain, synaptic extracts, total placenta and placental membrane vesicles in exposed cases vs. gestational age matched controls. Developmental expression of DING protein levels were quantified by western blot assay using the Odyssey® CLx Imaging System. Transcriptomic microarrays in human cells induced by human DING protein were performed. Results: pDING was developmentally expressed in brain and placenta tissues. Membrane fractions (placental vesicles and synaptic extracts) contained only the low molecular 37 kDa pDING isoform. Multiple DING isoforms were detected in total placenta and brain lysates. EtOH exposure was associated with increased placental pDING protein levels levels in the 1st trimester (Tri, ↑2.6 fold), and decreased pDING in the 2nd and 3rd TRI (↓2.6 and 4.0 fold). SSRI exposure with or without EtOH was associated with reduced placenta vesicle pDING (↓1.5 folds). In synaptic extracts, both EtOH and SSRI exposure were associated with reduced pDING (↓5.2; ↓1.1 fold respectively). Transcriptomics profiles for human DING revealed activation of several signaling pathways including cell survival, cell viability (↑5.4 fold) and inflammatory response, and identified several upstream regulators, including NUPR1, that empowers cells with resistance to the stress induced by a change in their microenvironment (↑5.6 fold). Conclusions: The 37kDa isoform of pDING, a key neuroprotective protein, is developmentally expressed in membrane extracts from human placenta and human fetal brain. Maternal EtOH and/or SSRI exposure is associated with decreased pDING levels, especially in the 2nd and 3rd trimesters, key periods of human cortical development. Our work supports a potential therapeutic/neuroprotective role of drug targets that result in increased pDING expression or in exogenous pDING in pregnancies at risk for FAS.

Disclosures: **N. Darbinian:** None. **N. Merabova:** None. **G. Tatevosian:** None. **M. Alamgir:** None. **E. Chabriere:** None. **L. Goetzl:** None.

Poster

689. Neurotoxicity: Protective Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 689.30/K23

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

Support: BMRC-13/1/96/19/691

SHF/FG635S/2014

Title: Hyperhomocysteinemia increases ER stress and Impairs autophagic flux in murine therapy brain that is reversible with vitamin therapy

Authors: *M. TRIPATHI[1], C. ZHANG[1], B. K. SINGH[2], R. A. SINHA[2], K. MOE[3], D. DE SILVA[1], P. M. YEN[2];
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Abstract: Introduction: Elevated serum level of Hcy, hyperhomocysteinemia (HHcy), is a strong independent risk factor for ischemic stroke. Several vitamin intervention studies have shown their efficacy in lowering HHcy. Epidemiological studies have that increased dietary intake of antioxidants/vitamins prevented and decreased the extent of ischemic brain injury. However the causal mechanism remains elusive. Methodology: We used a diet-induced model (32 mice; C57BL6) and HHcy was confirmed in the serum. After 12 weeks, 16 mice were sacrificed (8 control and 8 HHcy) and remaining 16 mice were put on diet enriched in vitamin B12 and folate and were also sacrificed after 12 weeks. Brain tissue samples were collected from both the sacrifice points and were used for protein and RNA analysis. For *in vitro* studies, SH-SY5Y neuroblastoma and astrocytes were taken and were treated with varying concentrations of Hcy as well as vitamin therapy. Results: We observed that HHcy led to in an increase in ER stress, as evidenced by increase in CHOP, ATF-6, p-eIF2α and p-ERK. Also we observed that HHcy caused a significant increase in ubiquitination. We also examined expression of autophagic markers LC3B-II and p62 and found significant inhibition of autophagy in HHCy brain samples. In contrast, in samples from vitamin-supplemented HHCy mice, autophagy induction was observed along with restoration in ER stress markers to normal levels (CHOP, ATF-6 p-eIF2α and p-ERK). In addition phosphorylation of AKT and target of rapamycin (mTOR) and were found to be significantly increased in HHcy brain as well as SH-SY5Y cells consistent with decreased autophagy. Vitamin therapy also reversed these changes in AKT and mTOR. Conclusion: Results from this study suggest that HHcy increased ER stress in murine brain, SH-SY5Yand astrocytes. We also found that increased ER stress was associated with down-regulation of autophagic flux. Interestingly, vitamin (B12 and Folate) therapy not only rescued the block in autophagy induced by HHcy but also prevented ER-stress. Collectively, these results show for the first time that HHcy induces ER stress in the brain that was reversed by vitamin therapy. They also suggest that vitamin therapy might reduce the extent of brain injury during a stroke.

Disclosures: **M. Tripathi:** None. **C. Zhang:** None. **B.K. Singh:** None. **R.A. Sinha:** None. **K. Moe:** None. **D. De Silva:** None. **P.M. Yen:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.01/K24

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

Support: P50MH103222

Title: Inhibition of quinolinate neosynthesis causes a rapid increase in extracellular kynurenic acid levels in the rat brain

Authors: *H.-Q. WU[1], G. COSTANTINO[2], F. M. NOTARANGELO[1], R. SCHWARCZ[1]; [1]Maryland Psych Res. Ctr, Univ. Maryland Sch. Med., Baltimore, MD; [2]Dept. di Farmacia, Universitaì degli Studi di Parma, Parma, Italy

Abstract: Quinolinic acid (QUIN), an excitotoxic NMDA receptor agonist and metabolite of the kynurenine pathway (KP) of tryptophan degradation, is present in the mammalian brain and may be causally involved in the pathophysiology of major neurodegenerative and psychiatric diseases. In the brain as elsewhere, QUIN is formed from its immediate bioprecursor 3-hydroxyanthranilic acid (3-HANA) by 3-hydroxyanthranilic acid 3,4-dioxygenase (3-HAO), and newly formed QUIN can be readily recovered from the extracellular compartment using *in vivo* microdialysis (Brain Res. Bull., 33: 513-516, 1994). The present study used this methodology to examine possible biochemical relationships between QUIN and kynurenic acid (KYNA), a neuroprotective tryptophan metabolite, which is synthesized by irreversible transamination of kynurenine by kynurenine aminotransferases (KATs) in a competing arm of the KP (Nat. Rev. Neurosci., 13: 465-477, 2012). Interestingly, applied by reverse dialysis to the medial prefrontal cortex of adult rats, 3-HANA (30 μM) resulted in a 201 ± 9% increase in extracellular KYNA levels (to 4.8 ± 0.3 nM) after 2 h (n=6). This KYNA elevation was totally abolished when the new, selective 3-HAO inhibitor 2-aminonicotinic acid 1-oxide (UPAR-12; J. Med. Chem., 56: 9482-9495, 2013) (100 μM) was co-infused with 3-HANA (30 μM) (n=6). To assess the possible role of NMDA receptor stimulation, and thus early, QUIN-induced excitotoxic events, 3-HANA (30 μM) was co-infused with the selective receptor antagonist (-)-2-amino-7-phosphonoheptanoic acid (50 μM). This co-treatment dramatically reduced the elevation of extracellular KYNA (to 115 ± 13% of baseline values; n=4). Co-infusion of 3-HANA (30 μM) with the non-specific KAT inhibitor aminooxyacetic acid (300 μM; n=3) or with the specific KAT II inhibitor (S)-(4-ethylsulfonyl)benzoylalanine (3 mM; n=4) also attenuated the 3-HANA-induced KYNA elevation (to 93 ± 10% and 121 ± 12% of baseline, respectively), indicating that enzymatic de novo formation from kynurenine plays a major role in the increases seen in extracellular KYNA. Finally, because the possibility that non-enzymatic oxidation of kynurenine to KYNA may be involved, the antioxidant glutathione (10 mM) was co-infused with 3-HANA (30 μM) (n=5). This treatment also attenuated the elevation in KYNA (to 151 ± 17% of

baseline). Taken together, these results reveal a heretofore unknown rapid stimulatory effect of relatively moderate increases in QUIN production on extracellular KYNA in the brain. Regardless of its underlying mechanism, this effect may have significant ramifications for brain physiology and pathology.

Disclosures: H. Wu: None. **G. Costantino:** None. **F.M. Notarangelo:** None. **R. Schwarcz:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.02/K25

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

Support: CB 183867

CB 180851

Title: Effect of kynurenines on copper toxicity in primary cultured astrocites

Authors: *D. RAMÍREZ ORTEGA[1], B. PINEDA[2], D. GONZÁLEZ ESQUIVEL[3], C. RÍOS[3], V. PÉREZ DE LA CRUZ[3];
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Abstract: Copper is a heavy metal and an integral component of various enzymes. It is necessary for mitochondrial respiration and other biological functions; however, excess copper is neurotoxic and has been implicated with neurodegenerative diseases as Alzheimer. This metal is able to modify the cellular redox environment, which can influence various cellular functions. On another hand, tryptophan degradation through kynurenine pathway is also modulated by the redox environment and produces some metabolites with redox properties as L-kynurenine (L-KYN), kynurenic acid, 3-hydroxykynurenine (3-HK) and 3-hydroxyantrhanilic acid (3-HANA). The imbalance on the production of these kynurenines is related with some pathologies. The aim of this study was to evaluate the effect of these kynurenines on the cupper toxicity through MTT reduction, ROS production and mitochondrial membrane potential on primary cultured astrocytes. First, we evaluated the CuSO4 (0-500 μM) effect on mitochondrial function by MTT assay. Then was evaluated the effect of the co-incubation of CuSO4 whit L-KYN (10 μM), 3-HK and 3-HANA (100 μM) on mitochondrial membrane potential, cellular function (MTT

reduction) and cell death by propidium iodide assay. Our results showed that CuSO4 decreased MTT reduction in a concentration-dependent manner and the co-incubation with 3-HK and 3-HANA potentiated this effect. CuSO4 decrease mitochondrial membrane potential and the co-incubation with 3-HK and 3-HANA potentiated this parameter as well as the cell death. While the co-incubation CuSO4 with L-KYN attenuated partially the decrease on mitochondrial membrane potential but also increased the cell death. Data suggest that these kynurenines increased the cell vulnerability induced by CuSO4.

Disclosures: **D. Ramírez Ortega:** None. **B. Pineda:** None. **D. González Esquivel:** None. **C. Ríos:** None. **V. Pérez de la Cruz:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.03/K26

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

Support: CHDI Foundation

Title: Kynurenine pathway metabolites modulate neurodegeneration and related phenotypes in *Drosophila*

Authors: C. BREDA[1], *K. V. SATHYASAIKUMAR[2], F. M. NOTARANGELO[2], S. S. IDRISSI[1], J. G. ESTRAINERO[1], G. G. L. MOORE[1], E. W. GREEN[1], C. P. KYRIACOU[1], R. SCHWARCZ[2], F. GIORGINI[1];
[1]Dept. of Genet., Univ. of Leicester, Leicester, United Kingdom; [2]Maryland Psychiatric Res. Center, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Metabolites of the kynurenine pathway (KP) of tryptophan (TRP) degradation have been implicated in the pathophysiology of Huntington's (HD), Parkinson's (PD) and Alzheimer's (AD) diseases. In a *Drosophila* model of HD, genetic inhibition of two pivotal KP enzymes, kynurenine 3-monooxygenase (KMO) and tryptophan 2,3-dioxygenase (TDO), normalizes KP imbalances and rescues neurodegeneration (Campesan et al., 2011). In support of these findings, van der Goot et al. (2012) showed that genetic down-regulation of TDO decreases toxicity in a worm model of PD. Here we sought to characterize the neuroprotection conferred by TRP treatment and TDO inhibition in HD flies, and to extend our analyses of the neuroprotective potential of KP manipulation to fly models of PD and AD. To this end, we analyzed rhabdomere neurodegeneration, climbing performance and longevity in PD and AD flies. HD flies fed with

TRP showed a dose-dependent reduction of neurodegeneration compared to controls ($p<0.001$). Further, an increased kynurenic acid/3-hydroxykynurenine (KYNA/3-HK) ratio was observed in these flies ($p<0.001$), suggesting that neuroprotection is due at least in part to increased levels of KYNA. Notably, 3-HK feeding abrogated neuroprotection in TDO-deficient HD flies, which normally exhibit reduced levels of 3-HK ($p<0.01$). Flies were also humanized to investigate whether quinolinic acid (QUIN), which is not present in flies, could cause toxicity in this model. QUIN feeding enhanced neurodegeneration in HD flies and significantly abolished the neuroprotection conferred by KMO inhibition, indicating that QUIN can cause toxicity independently of 3-HK. Moreover, a *Drosophila* line carrying a transgene encoding for human kynurenine aminotransferase (hKAT), the biosynthetic enzyme of KYNA, reduced neurodegeneration in HD flies ($p<0.001$), indicating that increasing KYNA levels, and an increase in the ratio of KYNA/3-HK in these flies ($p<0.001$) is neuroprotective. The efficacy of KMO and TDO was also tested in *Drosophila* models of PD and AD. Transgenic fly lines expressing human αSyn (PD model) or Aβ42Arc (AD model) were used. In both models, gene silencing of TDO or KMO increased the median lifespan of flies ($p<0.001$) and improved the climbing performance ($p<0.001$). Finally, pharmacological inhibition of TDO with the inhibitor 680C91 was protective in all the above models. Taken together, these studies in flies support the concept that targeted manipulation of KP metabolism constitutes a viable therapeutic strategy in several neurodegenerative diseases.

Disclosures: C. Breda: None. **K.V. Sathyasaikumar:** None. **F.M. Notarangelo:** None. **S.S. Idrissi:** None. **J.G. Estrainero:** None. **G.G.L. Moore:** None. **E.W. Green:** None. **C.P. Kyriacou:** None. **R. Schwarcz:** None. **F. Giorgini:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.04/K27

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

Support: NSF HRD-1137725

Title: Dibutyl phthalate affects Synaptic growth and stability at the *Drosophila* NMJ

Authors: *K. M. DE LEON[1], W. AQUINO[2], B. MARIE[2];
[1]Biol., [2]Inst. of Neurobio., San Juan, Puerto Rico

Abstract: Dibutyl phthalate (DBP) is a contaminant found ubiquitously in the environment that could therefore affect multiple organisms. Nevertheless, little is known about the effects of DBP on the nervous system. Studies have shown that DBP acts as a teratogen and as an endocrine disruptor. In addition, a recent study has linked DBP to neurotoxicity in neonatal rats. In the present study, we address the effect of DBP at the neuromuscular junction (NMJ). We reared animals in contaminated food and tested the effect of DBP on the synapse structure and morphology. We expressed the concentration of DBP present in the food as a function of the maximum contaminant level (MCL) allowed in public drinking water system (defined by the Environmental Protection Agency). We characterized a variety of synaptic markers: the vesicle marker synapsin, the microtubule associated protein Futsch (MAP1B homolog), the active zone associated structural protein Bruchpilot (CAST homolog), the postsynaptic marker discs large (Dlg; PSD-95 homolog) and the presynaptic membrane marker HRP. The animals that were reared in a milieu containing 10 times the MCL for DBP, presented a reduction in synaptic growth. Indeed, synapses from contaminated milieu showed a growth that was 80% of the control synapses. In addition, the animals reared in contaminated milieu showed synaptic retractions. These retractions are defined by the presence of postsynaptic markers and the absence of a subset of presynaptic markers. In animals reared in a milieu containing 10 times MCL for DBP, 50% of the synapses showed a retraction phenotype (boutons lacking synapsin staining) and these retractions showed a severity of 6 boutons on average. We also observed that the animals exposed to DBP have boutons deficient in MAP1B and CAST, both hallmarks of synaptic retractions. This effect was found to be dose dependent. When the animals were reared in a milieu containing 5 times the MCL for DBP, the synaptic growth was normal and the frequency of the retractions was reduced to 30%. Nevertheless, at this concentration, the retractions remained as severe. When the animals were reared in a milieu containing the MCL for DBP, there was normal synaptic growth, with no retraction phenotype. In addition, it seems that only chronic exposure to DBP is deleterious to synaptic growth and stability, as acute exposure did not affect synaptic growth or retractions. We are now focusing on the consequences of chronic exposure to DBP on synaptic release and plasticity.

Disclosures: **K.M. De Leon:** None. **W. Aquino:** None. **B. Marie:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.05/K28

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

Support: CONACYT (CB16782 & #120452)

PROMEP (103.5/10/7697)

FAI-UASLP (C12-FAI-03-62.62)

Title: Cytomegalovirus affects differentiation and function of neural stem cells upon infection

Authors: *H. M. GONZÁLEZ[1], A. CARRIZALES-HUERTA[2], M. JIMÉNEZ-CAPDEVILLE[1], D. NOYOLA[1], C. G. CASTILLO[1], A. MARTÍNEZ-SERRANO[3]; [1]Facultad De Medicina, U.A.S.L.P., San Luis Potosi, Mexico; [2]Facultad de Ciencias Químicas, Univ. Autónoma de San Luis Potosí, San Luis Potosí, Mexico; [3]Ctr. de Biología Mol. Severo Ochoa, Univ. Autónoma de Madrid, Madrid, Spain

Abstract: Cytomegalovirus (CMV) is the most common cause of congenital infection in developed countries and a major cause of developmental brain disorders in children, including mental retardation and hearing loss. Some *in vitro* models have demonstrated that neural precursor cells (NPCs) show the greatest susceptibility to CMV infection in developing brains. We sought to investigate the effects of CMV infection in human neural stem cells, in order to demonstrate that low virus concentrations can modify the correct function of these cells and then may have a significant impact in the developing brain. We established an *in vitro* model of CMV infection making use of an immortalized neural cell line, hNS-1 and the human CMV laboratory strain AD169. Cellular monolayers were infected at the multiplicity of infection of 0,1 and 0,01 plaque-forming units per cell. Viral infection was corroborated by detection of the CMV glycoprotein B using an indirect immunofluorescence assay. We induced infected hNS-1 to differentiate and evaluated the expression by immunocytochemistry of cellular markers of differentiation such as GFAP, MAP-2, nestin and vimentin, reflecting the presence of astrocytes, neurons and neural progenitors, respectively. Furthermore, the function of infected NPCs was assessed by measurement of the calcium influx in response to some stimuli. Our results show that NPCs are permissive to infection and the cytopathic effect was extensive. The expression of the marker for astrocytes decreased and the other proteins were unchanged. For the neural function assays, the onset time for some stimuli increased meanwhile the magnitude of the response was diminished. Our results suggest that CMV is able to produce morphological and functional changes in human neural stem cells and therefore alterations in the developing brain, even if low viral concentrations reach the central nervous system.

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Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.06/K29

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

Support: NIH R01GM089807

Title: Persistent systemic and brain inflammation and altered brain cholinergic system constituents in murine sepsis survivors

Authors: N. ZAGHLOUL[1], H. SILVERMAN[2], H. PATEL[2], S. VALDES FERRER[2], M. DANCHO[2], A. REGNIER-GOLANOV[3], P. OLOFSSON[2], *E. V. GOLANOV[4], C. METZ[2], M. AHMED[1], S. S. CHAVAN[2], K. J. TRACEY[2], V. A. PAVLOV[2];
[1]North Shore–LIJ Hlth. Syst., New Hyde Park, NY; [2]The Feinstein Inst. for Med. Res., Manhasset, NY; [3]Baylor Col. of Med., Houston, TX; [4]Neurosurg., Houston Methodist Hosp., Houston, TX

Abstract: Sepsis - a clinical syndrome associated with dysregulated immune responses is the most frequent cause of death in the intensive care units. Sepsis is associated with impaired brain function, which can be an independent predictor of mortality. Increased morbidity and cognitive impairment have also been reported in sepsis survivors. Providing insight into the brain alterations in sepsis survivors is important and may indicate novel therapeutic strategies. Here, we studied microglia activation and gene expression of cytokines and cholinergic system constituents in the cerebral cortex of mice - sepsis survivors, in parallel with peripheral (serum) cytokine analysis. Mice were subjected to cecal ligation and puncture (CLP) - a clinically relevant model of sepsis, which resulted in 50% mortality (vs. 100% survival in sham-operated mice) within 5 days. Murine sepsis survivors and sham controls were monitored for 9 additional days prior to utilizing them in the study. Iba1 protein immunostaining revealed marked morphological alterations in microglia - an amoeboid shape, indicative for microglia activation in mice sepsis-survivors. This microglial inflammatory phenotype was associated with significant upregulation of Iba1 gene expression. Cortex Il1b (a pro-inflammatory cytokine) mRNA expression was significantly increased ($P<0.01$) in mice sepsis-survivors. The increase in Il6 mRNA expression was not statistically significant ($P<0.08$). Ache (acetylcholine degrading enzyme) mRNA expression was increased ($P<0.03$), while Chat (acetylcholine biosynthesizing enzyme) mRNA expression was not significantly altered ($P<0.2$). In addition, nitrotyroisine protein immunostaining indicated increased nitrative stress in mice sepsis survivors. These brain alterations in mice - sepsis survivors were associated with higher serum IL-1β ($p<0.04$), IL-6 ($p<0.02$) and other cytokine levels. These results reveal a previously unrecognized persistent brain inflammatory activation in parallel with increased systemic cytokine levels in sepsis survivors. In addition, the increased brain Ache gene expression is of interest for further studying

of this enzyme (with an important role in cognition and in the regulation of inflammation) in the context of post-sepsis pathology. This study was funded in part by NIH/NIGMS

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Poster

690. Models of Neurodegeneration

Location: Hall A

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

Support: NIH Grant 1K99ES022992

NIH Grant EY011261

Title: The tadpole visual system as a model for assessing the effects of thyroid hormone disruption on brain development

Authors: *C. K. THOMPSON[1], M. TURKEN[2], K. D. MEDGYESY[3], H. T. CLINE[1]; [1]The Scripps Res. Inst., La Jolla, CA; [2]Univ. City High Sch., San Diego, CA; [3]UCSD, San Diego, CA

Abstract: Thyroid hormone (TH) plays an important role in brain development, and evidence shows that some compounds used in the production of industrial and consumer products can disrupt TH signaling, with severe consequences for behavior. How TH disruptors impact neural circuit development is still unclear, however. Tadpoles are useful models for better understanding endocrine disruption because they are acutely sensitive to changes in TH signaling. In addition, their external development allows for manipulation and observation of the early stages of brain development which are relatively inaccessible for study in mammalian systems. Here we show that visual system development in Xenopus laevis tadpoles is robustly affected by changes in TH signaling. Our data show that treatment with TH for 7 days induced vast changes in brain morphology, including regression of the telencephalon and expansion of the optic tectum volume. In addition, TH acts directly on the brain and the retina to significantly increase the rate of proliferation in the ventricular zone and ciliary margin, respectively. Using *in vivo* imaging, we found that TH treatment increased the rate of neuronal differentiation as well as

increased dendritic arborization. TH also increased the expression of TH sensitive genes. Triclosan, an anti-microbial agent used in a variety of consumer household products, may disrupt TH function. We are currently engaged in experiments testing the degree to which triclosan mimics TH to affect visual system development. Our preliminary data strongly suggest that triclosan does not induce TH-like changes that affect Xenopus tadpole visual system development. These experiments establish a baseline for the sensitivity of tadpole brain development to changes in TH signaling against which we will compare the effects of TH disruptors.

Disclosures: C.K. Thompson: None. **M. Turken:** None. **K.D. Medgyesy:** None. **H.T. Cline:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.08/K31

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

Title: An acute inhalation study on the effect of Hydrofluorocarbon 134a gas on the frontal cerebral cortex of albino wistar rats

Authors: *E. DIKE, M. N. WOGU, E. EKONG;
Univ. of Port-Harcourt, Port-Harcourt, Nigeria

Abstract: Incidence of accidental exposure to high concentration of refrigerant gases in factories, chemical industries and domestic houses are on the increase but the awareness on the health implications of the acute inhalation of these gases is not widespread such as its neuroanatomical basis. This study investigates the effect of acute HFC 134a gas inhalation on the weight, oxidative stress (Malondialdehyde level (MDA), Glutathione peroxidase ($GSHP_X$) level and histomorphology of the frontal cortex of cerebrum of albino wistar rats. In this study a total of 43 albino wistar rats weighing about 140-280g were used for the study. 15 out of the 43 rats were used for the Lethal Concentration 50 (LC50 test or limit study) and the remaining 28 for the test study, the LC50 for this study was found to be 44660ppm from which the test concentration was derived. The test study consists of 4 groups of 7 rats each. Group A was exposed to ½ of the LC50, Group B to 1/3 of LC50, Group C to 1/10 of LC50 and a control group. A whole body exposure system was used for the study. All groups were exposed to a single exposure to HFC 134a for 30 minutes inside an inhalation chamber except for the control group which were exposed to free air in the chamber for the same duration. The MDA, $GSHP_X$ level, body weight

and brain tissue samples were obtained from the animals immediately after exposure, 7 days after exposure and 14 days after exposure. From this study, it was noticed that there was significant weight reduction in exposed rats, serum level of MDA was significantly higher in rats immediately after exposure and 7 days after exposure which is indicative of increased oxidative stress while MDA level in day 14 was not significantly different from control group. There was profound distortion on the frontal cerebral cortex histomorphology of exposed rats especially, on the day of exposure and day 7 after exposure. There was no difference between the histomorphology of the frontal cortex of rats 14 days after exposure and in the control group. Thus it could be deduced that acute inhalation of HFC134a causes significant weight loss, oxidative stress and distortion in the histomorphology of the frontal lobe of cerebral cortex immediately after exposure and 7 days after exposure but these effects were alleviated on the 14th day after exposure. **Keywords:** HFC 134a, acute inhalation, cerebrum, Neurotoxicity.

Disclosures: **E. Dike:** None. **M.N. Wogu:** None. **E. Ekong:** None.

Poster

690. Models of Neurodegeneration

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

Support: NIAAA Grant R21 AA 021260-02

Title: Gender differences in binge alcohol-induced brain damage

Authors: ***M. E. MAYNARD**, C. R. ROBINSON, E. A. BARTON, J. L. LEASURE; Psychology, Univ. of Houston, Houston, TX

Abstract: Binge pattern drinking is the most common alcohol use disorder (AUD) and has been linked to severe neuropathology. Mounting evidence indicates that the brains of men and women may respond differently to binge alcohol consumption. Women appear more sensitive to the neurotoxic effects of alcohol, with cognitive and structural impairments emerging faster in women than men. Additionally, women with AUDs have higher rates of medical problems and a significantly higher death rate than men with AUDs. It is alarming then that rates of binge pattern consumption have been increasing at a much faster rate in women than men in the last ten years. The current study assessed gender differences in brain damage following binge alcohol exposure using a well-established rodent model. Adult female and male Long-Evans rats were gavaged with ethanol (25% w/v) in nutritionally complete diet every 8 hours for 4 days. Control

animals received isocaloric control diet. Stereological analysis of hippocampal dentate gyrus granule cells following 4 days of binge exposure revealed a significant 13% loss in female rats but no corresponding decrement in male rats. Both female and male binged hippocampi showed a significant decrease in proliferating (Ki67+) cells, thus, a lack of cell replacement cannot account for the decrement in granule neurons in females. To probe for binge-induced differences in trophic support, we performed Western blotting for brain-derived neurotrophic factor (BDNF), its receptor (trkB) and insulin-like growth factor receptor (IGFR) in male and female rats sacrificed after 1, 2 or 4 days of binge exposure. Male, but not female hippocampi showed an increase in IGFR after 1 day of binge exposure. Female, but not male hippocampi showed a decrease in trkB receptors after 2 or 4 days of binge exposure. Females also showed an increase in BDNF protein after 4 days. Taken together, our results reflect an animal model of female brain susceptibility to binge alcohol damage. This enhanced vulnerability may be due to a decrease in available trophic support during the binge, and a delayed compensatory increase that occurs too late to attenuate damage. Ongoing studies are investigating other potential mechanisms underlying female brain susceptibility to binge alcohol, including differential inflammatory responses and oxidative stress. Finally, gender differences in binge-induced cognitive impairments are being addressed. To conclude, the present data indicate a potential mechanism underlying female brain susceptibility to alcohol-induced damage.

Disclosures: **M.E. Maynard:** None. **C.R. Robinson:** None. **E.A. Barton:** None. **J.L. Leasure:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.10/K33

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

Support: Pioneer Research Center Program through the National Research Foundation of Korea 2012-0009521

Title: Apomorphine attenuate ethanol-induced neurodegeneration in adult rat cortex

Authors: ***M.-H. JO**, H. BADSHSH, N. MUHAMMAD, M.-J. KIM, M.-O. KIM; Gyeongsang Natl. Univ., Jin-ju, Korea, Republic of

Abstract: Apomorphine is a dopamine D1/D2 receptor agonist, therapeutically used for Parkinson disease, has been found to be potent antioxidant and prevents free radical reaction in

rat brain mitochondrial fraction. Alcohol, being a neurotoxic agent causing neurodegeneration possibly through free radical generation. Here, we investigated apomorphine has antioxidant potential against ethanol induced neurodegeneration in cortex of adult rats. Ethanol induced apoptotic neurodegeneration was determined by the suppression of Bcl-2, induction of Bax, release of cytochrome C and activation of caspase-9 and caspase-3. Moreover, ethanol elevated level of cleaved PARP-1 indicating exaggerated neuronal DNA damage. Our results pointed out a dose dependent neuroprotective effect of apomorphine reversing the ethanol induced apoptotic trend as observed by the increased expression of Bcl-2, downregulation of Bax, inhibition of mitochondrial cytochrome C release and inhibition of activated caspase-9 and caspase-3. Immunohistochemical analysis and Nissl staining also revealed neuronal viability maintained by apomorphine after ethanol induced neuronal cell death. Furthermore, apomorphine decreased the ethanol induced neuronal damage in a dose dependent manner showing more neuronal viability at dose of 5mg/kg than that of 1mg/kg. Our finding suggest that apomorphine attenuated ethanol induced neuronal cell loss in the adult rat cortex and has a more effective neuroprotective behavior at dose of 5mg/kg than that of 1mg/kg. Finally we can conclude that apomorphine has effective therapeutic potential to protect brain against ethanol induced neurotoxicity.

Disclosures: **M. Jo:** None. **H. Badshsh:** None. **N. Muhammad:** None. **M. Kim:** None. **M. Kim:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.11/K34

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

Title: The potential role of Sigma-1 receptor chaperone in the genesis of autophagosome in neuronal cells

Authors: *T.-Y. WENG, T.-P. SU, S.-Y. TSAI;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: The Sigma-1 receptor (Sig-1R) is an endoplasmic reticulum (ER) chaperone protein that involves in the neuronal homeostasis. Silencing of Sig-1R in primary neurons has been shown to induce ER stress, ROS production and mitochondrial dysfunctions. These cellular responses are closely related to the autophagy response when cells strive for survival. We thus test the hypothesis that Sig-1R knockout (KO) neurons are prone to an increased autophagy

response to compensate for cellular survival and that Sig-1R may relate to the genesis of autophagosome. Our preliminary data show that the preautophagosomal marker LC3-I is enhanced in the Sig-1R KO primary neurons when compared to that from wild type neurons. This result suggests that without Sig-1R the formation of autophagosome is increased in cells to compensate for survival. We next used the autophagosome inhibitor chloroquine (CQ), which accumulates in the lysosome and blocks the cellular process of autophagy, to examine the role of Sig-1R as such. We found that in the presence of CQ the autophagy marker LC3-II and its cargo protein p62 levels were reduced in Sig-1R KO primary neurons when compared to wild type neurons. Further, the reduced LC3-II was also observed in Sig-1R silenced Neuro-2a cells under serum starvation conditions. Those data suggests that the process of autophagy is hindered in the Sig-1R KO primary cortical neurons or Sig-1R silenced cells. Additionally, it is known that autophagosomes and endosomes will fuse and converge. We found that the early endosome marker EEA1 was reduced in Sig-1R KO or silenced cells in the presence of CQ, further implicating Sig-1R as a player in the initial phase of the autophagy formation. No differences in the level of autophagy-related (Atg) proteins, 5 and 12 conjugation systems, were observed. Giving that the majority of Sig1R resides in the ER-mitochondrion interface which plays pivotal roles in vesicle transport pathways to form autophagosome, we will focus on the exact functions of Sig-1R in the endosomal-autophagy pathway in the future.

Disclosures: **T. Weng:** None. **T. Su:** None. **S. Tsai:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

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Program#/Poster#: 690.12/K35

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

Title: Antiallodynic interaction of dexmedetomidine plus morphine in cisplatin-induced neuropathic pain in rat

Authors: *A. ZUÑIGA[1,2], J. REYES GARCÍA[2], F. FLORES MURRIETA[2], H. ROCHA GONZALEZ[2];
[1]INER, MEXICO DF, Mexico; [2]INSTITUTO POLITECNICO NACIONAL, Mexico df, Mexico

Abstract: Clinical use of anti-neoplastic drugs is associated with the development of numerous adverse effects that many patients find intolerable, including peripheral neuropathy. Cisplatin, an effective anti-neoplastic agent in the treatment of solid tumors, produces a dose-limiting painful peripheral neurophathy in a clinically significant number of cancer patients. The aim of the

present study was to investigate the possible antiallodynic effect of systemic administration of dexmedetomidine and morphine either alone or in combination in an animal model of neuropathic pain evoked by cisplatin. Mechanical allodynia was measured by using von Frey filaments in the hindpaw. Male Wistar rats, weighting 180-220 g, were treated with cisplatin administered intraperitoneally three times a week (Monday, Wednesday, and Friday) at a dose of 0.1 mg/ 100 g weight. Dexmedetomidine (10, 30, 56 and 100 µg/kg), morphine (0.1, 0.3, 3 and 5.6 mg/kg) or equieffective doses of the combination in a ratio|1:1 were administered to obtain the 40% experimental effective doses (ED_{40}) for each drug and for the combination. Isobolographic analysis was performed to examine the interaction. Dexmedetomidine (ED_{40} = 33.41 +/- 3.5 µg/kg), morphine (ED_{40} = 3.2 +/- 0.7 mg/kg) and fixed-dose ratio dexmedetomidine-morphine combinations showed dose-dependently anti-allodinical effect. Theoretical ED_{40} value for the combination estimated from the isobologram was 2.05 +/- 0.3 mg/kg, whereas that experimental ED_{40} value was 1.20 +/-0.3 mg/kg. Results indicate that i.p. administration of dexmedetomidine plus morphine can interact synergistically in the neuropathic pain model and suggest the use of this combination to relieve pain evoked by anti-neoplastic agents in humans. **Keywords:** Antineoplastic drugs, Cisplatin, Neurophaty, Dexmedetomidine, Morphine.

Disclosures: A. Zuñiga: None. **J. Reyes García:** None. **F. Flores Murrieta:** None. **H. Rocha Gonzalez:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

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

Support: INP Grant 123240.1

GRESMEX Grant 2015

Title: Intracerebroventricular injection of functionalized titanium dioxide nanoparticles (NBelyax®) and electroencephalographic activity in freely moving rats

Authors: *V. M. MAGDALENO-MADRIGAL[1], G. CONTRERAS-MURILLO[1], R. FERNÁNDEZ-MAS[1], P. ARTEAGA-LÓPEZ[2], G. LEÓN-GUTIÉRREZ[2], L. ALBARRÁN[2], S. LEÓN-GUTIÉRREZ[2], E. GONZÁLEZ-TRUJANO[1];

[1]Inst. Nacional De Psiquiatría Ramón De La Fuente Muñiz, Ciudad De México, Mexico; [2]GRESMEX S.A. de C.V., Ciudad de México, Mexico

Abstract: Titanium dioxide nanoparticles (TiO2 NPs) are now in daily in a great variety of home use products, as NBelyax® a clean use home product. However, the effects of TiO2 NPs on the central nervous system are still unclear. The electroencephalographic (EEG) and background EEG power spectral analysis is a strategy to investigate and may be correlated with neurological disorders. The aim of this study was to determine if TiO2 NPs functionalized with biological molecules (NBelyax®) are able to modify the EEG activity in freely moving rats. Wistar male rats (280-320 g) were implanted with two guide-cannulas in both lateral ventricules (AP -0.5; L 1.0; H 3.0). Also both frontal, both parietal and both occipital cortices were implanted to EEG recording. Acute intracerebroventricular injection (ICV) to deliver 1 microliter/1 min of functionalized TiO2 NPs (NBelyax®) by ventricule was performed. All animals were subject to 1 h of EEG recording during five days and were compared with themselves EEG baseline obtained before of ICV of functionalized TiO2 NPs. The EEG analysis of cortical areas showed that the functionalized TiO2 NPs (NBelyax®) did not modify at 0-4, 4-10, 10-30 and 35-55 Hz bandwitdh frequencies and did not show adverse effects on the spontaneous motor activity of rats. These preliminary results suggest that functionalized with biological molecules the TiO2 NPs (NBelyax®) may not produce adverse effects on EEG activity under these conditions. In addition, more investigations are necessary to determine the chronic effects of TiO2 NPs (Nbelyax®) on EEG and spontaneous behavior of rats.

Disclosures: V.M. Magdaleno-Madrigal: None. **G. Contreras-Murillo:** None. **R. Fernández-Mas:** None. **P. Arteaga-López:** None. **G. León-Gutiérrez:** None. **L. Albarrán:** None. **S. León-Gutiérrez:** None. **E. González-Trujano:** None.

Poster

690. Models of Neurodegeneration

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

Support: NIH: K01AG044490

NIH: ES021243

NIH: ES024233

NIH: AG037919

NIH: AG037481

DOD: W81XWH-13-1-0384

Title: H3K9 acetylation profile and behavioral changes in response to arsenic exposure

Authors: *N. F. FITZ, K. NAM, A. MOUNIER, E. L. CASTRANIO, R. KOLDAMOVA, I. LEFTEROV;
Envrn. and Occup. Hlth., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Alzheimer's disease is characterized by a complex cascade of pathological hallmarks which are affected by different genes and environmental factors, as well as gene-gene and gene-environment interactions. Dietary patterns, exposure to environmental toxicants, intellectual and physical activity, as well as cardiovascular disease and Type 2 Diabetes, display some of the highest AD association. The molecular mechanisms of these factors underlying the increased susceptibility to, earlier development or aggravated course of the disease remain poorly understood. Molecular links between certain environmental exposures/factors and AD could result in altered regulatory and metabolic pathways due to dysregulation in transcription factor binding, or epigenetic changes known to influence synaptic transmission, memory and cognitive performance. Previously we showed that Early Growth Response 1 (EGR1) transcription factor regulates the constitutive expression of genes involved in vesicular transport and synaptic transmission that may be critical for memory and cognition. Here we demonstrate changes in H3K9 acetylation profile in the proximal promoter of mouse Egr1 in response to prenatal arsenic exposure and normal diet. We also found a significant decrease in the levels of Egr1 mRNAs in the brain of offspring following in-utero exposure, as well as in 2 month old mice exposed to human relevant 100 parts/billion (100 μg/L) arsenic in drinking water. These genomic changes were related to diminished memory function as tested by contextual fear conditioning. This illustrates the potential of environmental toxicants to impact molecular mechanisms also affected by AD possibly aggravating the course of the disease.

Disclosures: **N.F. fitz:** None. **K. Nam:** None. **A. Mounier:** None. **E.L. Castranio:** None. **R. Koldamova:** None. **I. Lefterov:** None.

Poster

690. Models of Neurodegeneration

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Program#/Poster#: 690.15/K38

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

Support: Allen and Company

The Marcus Foundation

The Stein Family Research Fund

Title: Therapeutic ionizing radiation-induced cognitive deficits: Modulation by the neurosteroid progesterone

Authors: *F. ATIF, S. YOUSUF, J. WANG, D. G. STEIN;
Emergency Med., Emory Univ., Atlanta, GA

Abstract: Despite continuous improvements, CNS toxicity of cancer treatments remains an important issue and a major cause of morbidity in cancer patients. The brain is reported to be susceptible to both high and low doses of ionizing radiation (IR), but whereas high-dose radiation exposure may lead to tissue damage, low-dose radiation exposure insidiously affects cognitive functions without causing noticeable tissue injury. We examined the neuroprotective effects of the neurosteroid progesterone (PROG) against IR-induced cognitive deficits in mice. Male C57/BL mice (non-tumor-bearing; 5-6 weeks) were exposed to two different fractionated doses of IR (3 Gy x 3 and 3 Gy x 5). PROG (16 mg/kg; single injection daily) was given as a pre-treatment (starting 3 days prior to IR treatment), concurrent treatment (during the IR treatment for 3 or 5 days), and 8 or 6 days post-IR treatment for a total of 14 days, with tapering during the last two days. Mice were tested for short- and long-term effects of IR and PROG on memory function on days 10 and 30 day after IR treatment. The spontaneous locomotor activity test revealed a significant ($P<0.001$) decrease in total distance travelled and movement time in both IR groups at both 10 and 30 days post-IR. PROG treatment showed significant ($P<0.01$) improvement at both time points. A significant ($P<0.001$) increase in resting time was observed in IR-treated groups at both 10 and 30 days post-IR. PROG treatment significantly ($P<0.01$) reduced the resting time in both IR groups at both time points. An elevated plus maze test was performed to measure anxiety-like behavior. A significant ($P<0.001$) increase in time spent in the open arms and the number of entries into the open arms was observed in both IR-treated groups at both 10 and 30 days post-IR. PROG treatment significantly ($P<0.01$) reduced the anxiety-like behavior in both IR groups at both time points. A Morris water maze test was performed between days 30-35 post-IR treatment. We observed significant ($P<0.001$) deficits in short- and long-term memory function in both IR groups. PROG treatment showed a significant ($P<0.001$) improvement in both short- and long-term memory function. There was no significant difference in the degree of deficit in any test between two doses of IR at either time point. Our data suggest that PROG could help to reduce the side effects of radiation, leading to a better quality of life for the cancer patient.

Disclosures: **F. Atif:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A US patent (# US 8,435,972 B2) was issued to FA and DGS on May 7, 2013 for the use of PROG and compositions related thereto for the treatment of neurogenic tumors.. **S. Yousuf:** None. **J. Wang:** None. **D.G. Stein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A US patent (# US 8,435,972 B2) was issued to FA and DGS on May 7, 2013 for the use of PROG and compositions related thereto for the treatment of neurogenic tumors..

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.16/K39

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

Support: CDMRP - GW080094

VA Merit - 1I01CX000469

CDMRP - GW1300045

Title: Omic analyses identify lipid and bioenergetic disturbances in the brains of a GWI mouse model at 16-months post-exposure

Authors: *L. ABDULLAH[1], J. E. EVANS[1], H. MONTAGUE[2], G. CRYNEN[1], J. REED[1], T. NGUYEN[1], M. HOWLAND[1], B. MOUZON[1], Z. ZAKIROVA[1], T. EMMERICH[1], D. PARIS[1], G. AIT-GHEZALA[1], M. MULLAN[1], C. BACHMEIER[1], F. CRAWFORD[1];
[1]Roskamp Inst., Sarasota, FL; [2]Roskamp Inst., sarasota, FL

Abstract: Veterans from the 1991 Gulf War (GW) suffer from Gulf War Illness (GWI), which remains untreatable due to its multi-symptom presentation. Since evidence suggests an involvement of the central nervous system (CNS) in GWI, we applied omics to identify biological disturbance within the brains of a mouse model of GWI. Barnes maze was performed to examine cognition and neuropathology to detect astroglia and microglia activation at 16-months following an acute exposure to GW agents, pyridostigmine-bromide (anti-nerve agent) and permethrin (a pesticide). Proteomics, lipidomics and metabolomics were performed on the brain tissue. Lipid transport was examined using an *in vitro* blood brain barrier (BBB) system. Protein changes corresponded with lipid transport and metabolism and bioenergetic disturbances

in exposed mice compared to controls. Phospholipid, citric acid cycle metabolites and lactate levels were reduced in GW agent exposed mice. A high ratio of arachidonic acid (AA) to docosahexaenonic acid (DHA) was detected in exposed mice. *In vitro* BBB studies suggested an imbalance in AA and DHA transport following exposure. In conclusion, lipid metabolism and transport and bioenergetic abnormalities are chronic CNS features of GWI in a mouse model, which could be targeted for the development of therapies for GWI.

Disclosures: L. Abdullah: None. **J.E. Evans:** None. **H. Montague:** None. **G. Crynen:** None. **J. Reed:** None. **T. Nguyen:** None. **M. Howland:** None. **B. Mouzon:** None. **Z. Zakirova:** None. **T. Emmerich:** None. **D. Paris:** None. **G. Ait-Ghezala:** None. **M. Mullan:** None. **C. Bachmeier:** None. **F. Crawford:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.17/K40

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

Support: OU College of pharmacy Research funds

Title: Neuronal and microglial cells differ in their sensitivity to glutamate toxicity

Authors: *H. O. AWWAD, S. DONG, A. EDWARDS;
Pharmaceut. Sciences, Col. of Pharm., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: Excessive glutamate release in traumatic brain injury and neurodegenerative diseases like Alzheimer's disease causes cellular damage known as excitotoxicity. This toxicity in neurons has the ability to enhance phosphorylation of the tau protein, a microtubule stabilizer, and leads to the destabilization of microtubules and cell apoptosis. Microglial cells are the resident immune cells in the central nervous system and similar to astrocytes, they play a neuroprotective role by converting glutamate into glutamine. Recent reports indicate the presence of glutamate receptors on microglia. Our aim from this study was to determine whether glutamate toxicity causes tau phosphorylation in the microglia, like it does in neurons. Human neuroblastoma SH-SY5Y and microglial murine BV2 cells were treated with high concentrations of glutamate (5, 10, 15, 25 and 50mM) for 20-24 hours. Cellular morphology and cell viability were observed, and cell lysates were collected to determine tau phosphorylation at serine 404. Toxic concentrations of glutamate decreased cell viability in both cell lines using an MTT assay. However, SH-SY5Y cell lines were more sensitive, showing a significant decrease in cell

viability at both 25 and 50 mM glutamate whereas BV2 cells only showed a decrease in cell viability at 50 mM glutamate. Tau phosphorylation immunoblotting indicates that tau phosphorylation at serine 404 occurred at 5 and 10 mM Glutamate concentrations in SH-SY5Y and not in microglial BV2 cells (n=2-4). This study suggests that the lack of tau phosphorylation in microglial cells following glutamate toxicity may explain another mechanism by which microglial cells could survive harsher conditions following a brain injury than neurons. Understanding the underlying neurobiology may shed light on potential neuroprotective mechanisms and therapeutic targets against glutamate toxicity.

Disclosures: **H.O. Awwad:** None. **S. Dong:** None. **A. Edwards:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

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

Support: Center of Stroke Research Berlin (01 EO 0801)

Volkswagen foundation (Lichtenberg program)

Cluster of Excellence NeuroCure (Exc 257)

Title: Targeting chemobrain: a novel mechanism to prevent chemotherapy induced cognitive deficits in C57Bl/6 mice

Authors: *P. HUEHNCHEN, W. BOEHMERLE, M. ENDRES;
Charite Universitätsmedizin Berlin, Berlin, Germany

Abstract: Neurotoxicity to the peripheral and central nervous system caused by chemotherapy present a yet unsolved medical problem. Especially post-chemotherapy cognitive impairment (PCCI) or “chemobrain” has become a growing concern to cancer patients worldwide. Research on the topic is relatively scarce and the pathomechanisms involved poorly understood. The goal of this study was to characterize behavioral as well as histological changes induced by paclitaxel therapy in mice and to establish a preventive treatment based on the underlying pathophysiological processes. Adult C57Bl/6 mice were injected with a total of 12 × 20 mg/kg bodyweight paclitaxel or vehicle respectively over the course of 4 weeks 3 times a week (dose-dense therapy). The treatment was in general well tolerated with limited weight loss. Animals receiving paclitaxel developed an axonal sensory polyneuropathy as previously described. In

addition, using the Morris water maze we observed impaired spatial learning and memory after paclitaxel treatment, but no alterations regarding motor performance or changes of affective behaviors or anxiety. Histological assessment revealed a decrease in hippocampal neuroneogenesis in paclitaxel-treated animals with reduced numbers of newly generated doublecortin-positive and NeuN-positive cells. Evaluation of pharmacokinetics showed nanomolar paclitaxel concentrations in the hippocampus up to 2 hours post injection. Comparable amounts of paclitaxel induced apoptotic cell death in cultured adult mouse neural stem cells (NSCs) via a calcium and calpain mediated mechanism. The observed molecular effects were distinct from the cytostatic effect of paclitaxel and could be specifically inhibited by lithium (Li+) *in vitro*. Subsequently, we tested co-treatment of mice with Li+, which prevented paclitaxel-induced changes in spatial learning as well as peripheral neuropathy and normalized neurogenesis in the dentate gyrus. Taken together, this study uses a mouse model of dose-dense paclitaxel therapy to characterize pharmacokinetics, behavioral and histological alterations as well as a putative molecular mechanism underlying paclitaxel-induced PCCI. Based on these results, we established a strategy to prevent neurotoxic side effects of paclitaxel-therapy in the central and peripheral nervous system.

Disclosures: **P. Huehnchen:** None. **W. Boehmerle:** None. **M. Endres:** None.

Poster

690. Models of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 690.19/K42

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

Title: Immunohistochemical study of nrf2-antioxidant response element as of oxidative stress induced by cadmium in developing rats

Authors: *M. MENDEZ-ARMENTA, S. MONTES, D. JUÁREZ-REBOLLAR, C. NAVA-RUÍZ, A. SÁNCHEZ-GARCÍA, Y. HERAS-ROMERO, C. RIOS, A. DIAZ-RUÍZ;
Natl. Inst. Neurol Neurosurg., Mexico City, Mexico

Abstract: In developing animals, Cadmium (Cd) induces toxicity to many organs including brain. Reactive oxygen species (ROS) are often implicated in Cd-inducedtoxicity and it has been clearly demonstrated that oxidative stress interferes with the expression of genes as well as transcriptional factors such asNrf2-dependent Antioxidant Response Element (Nrf2-ARE). Cd-generated oxidative stress and elevatedNrf2 activity have been reported *in vitro* and *in situ* cells. In this study we evaluated the morphological changes and the expression pattern ofNrf2 and

correlated them with the Cd concentrations in different ages of developing rats in heart, lung, kidney, liver, and brain. The Cd content in different organs of rats treated with the metal was increased in all ages assayed. Comparatively, lower Cd brain levels were found in rats intoxicated at the age of 12 days, then pups treated at 5, 10, or 15 days old, at the same metal dose. No evident changes, as a consequence of cadmium exposure, were evident in the morphological analysis in any of the ages assayed. However, Nrf2-ARE immunoreactivity was observed in 15-day-old rats exposed to Cd. Our results support that fully developed blood-brain barrier is an important protector against Cd entrance to brain and that Nrf2 increased expression is a part of protective mechanism against cadmium-induced toxicity.

Disclosures: M. Mendez-Armenta: None. **S. Montes:** None. **D. Juárez-Rebollar:** None. **C. Nava-Ruíz:** None. **A. Sánchez-García:** None. **Y. Heras-Romero:** None. **C. Rios:** None. **A. Diaz-Ruíz:** None.

Poster

690. Models of Neurodegeneration

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

Support: Defense Threat Reduction Agency - Joint Science and Technology Office

Title: Chemical warfare agent-induced gene expression changes in blood and potential use as diagnostic biomarkers

Authors: *H. M. HOARD-FRUCHEY[1], C. C. ROTHWELL[1], A. A. MELBER[1], C. S. HOFMANN[1], K. D. MOTTER[1], J. W. SEKOWSKI[2];
[1]USAMRICD, Aberdeen Proving Ground, MD; [2]USAECBC, Aberdeen Proving Ground, MD

Abstract: Chemical warfare nerve agents (CWNAs) are a class of cholinesterase inhibitors that are extremely potent. The recent use of the organophosphate sarin in Syria reiterates the devastating effects that these agents can have on human health and mortality. These agents bind to and disrupt the function of acetylcholinesterase, resulting in the accumulation of the neurotransmitter acetylcholine and a variety of symptoms including miosis, excessive secretions, convulsions, seizure, and death. Point-of-care diagnostic tests to identify CWNA exposure and/or to evaluate treatment efficacy are currently unavailable. In this study, the mRNA expression changes induced by the CWNA VX in the blood were determined to initiate identification of potential biomarkers for diagnosis of CWNA exposure and indicators of treatment efficacy for

peripheral and central medical countermeasures. Adult male Sprague-Dawley rats were exposed to 0.4, 0.7, or 1.0 x LD_{50} VX (i.v.), and trunk blood was collected at 1, 2, 4, 8, or 24 hours post-exposure. Total RNA was isolated and processed for mRNA hybridization to Affymetrix GeneChip HT RG-230 PM for microarray analysis. Principal component analysis (PCA) identified VX dose and not time as the greatest source of variability within the dataset. Analysis of variance (ANOVA) was performed to identify nerve agent-induced changes in gene expression over time. Using the 1.0 x LD_{50} VX data, canonical pathways significantly affected were identified and compared across the five post-exposure time points. Six canonical pathways were common to all time points. However, 2-, 4-, and 8-hour time points displayed the largest number of common canonical pathways (26 pathways). Genes with altered expression within these common canonical pathways were identified as potential biomarkers for VX exposure. Thirty-one genes were identified as differentially expressed for at least three time points. These candidate mRNA biomarkers are being evaluated to down select an mRNA panel for further development. *Disclaimer: The views expressed 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 U.S. Army Edgewood Chemical Biological Center 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.*

Disclosures: H.M. Hoard-Fruchey: None. **C.C. Rothwell:** None. **A.A. Melber:** None. **C.S. Hofmann:** None. **K.D. Motter:** None. **J.W. Sekowski:** None.

Poster

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

Support: This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S & T Division.

Title: Insulin-like growth factor-1 accelerates recovery from botulinum neurotoxin-induced paralysis

Authors: *J. P. APLAND[1], C. H. PHUNG[1], E. J. GLOTFELTY[2], T. M. RUSSO[2], P. M. MCNUTT[2], M. ADLER[1];
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Abstract: Recovery from paralysis induced by botulinum neurotoxin serotype A (BoNT/A) is slow, requiring >90 days for restoration of muscle function and mass in the locally injected rat extensor digitorum longus (EDL) muscle. Several years may be required for complete recovery in severely intoxicated humans. In previous studies, we investigated the effects of local intramuscular injection of insulin-like growth factor-1 (IGF) on recovery from BoNT/A-induced paralysis. IGF was injected 1, 3, and 5 days after injection of BoNT/A into left EDL (LEDL) muscles of adult male rats and 3 times/week thereafter for 7, 14, 21, and 28 days. The right EDL muscle of each rat served as an uninjected control. A control group received BoNT/A injections into LEDL muscles at the same time as the experimental group, but no IGF injections. In the latest studies we injected IGF subcutaneously to more closely resemble the human dose paradigm. At the specified times after BoNT/A injections, *in situ* muscle tension measurements were performed, rats were euthanized, and EDL muscles were removed, weighed, and fixed for assessment of atrophy. Results indicate that LEDL muscle mass in rats injected with IGF recovered more rapidly than in rats injected with BoNT/A only. Muscle tensions in LEDL muscles of IGF-injected rats were also higher at all stimulus frequencies than in BoNT/A-only rats. In histological sections, the IGF-treated muscles exhibited significantly less injury and atrophy. Immunocytochemical techniques confirmed that BoNT/A intoxication elicited significant changes in muscle fiber diameter; these changes were rescued by IGF injections. IGF treatment furthermore reduced BoNT/A-induced axonal sprouting and preserved endplate organization. These results suggest that treatment with IGF may have therapeutic value in accelerating recovery from BoNT/A-induced paralysis. The experimental protocol was approved by the Animal Care and Use Committee at the USAMRICD 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. The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

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Poster

690. Models of Neurodegeneration

Location: Hall A

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The University Grants Commission, New Delhi, India (HSS/AS)

Title: Repeated depressive stress exacerbates amyloid-beta peptide infusion induced Alzheimer's disease brain pathology. Neuroprotective effects of PLGA-NPs loaded cerebrolysin

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Abstract: Our previous studies show that stressful situations alone could induce breakdown of the blood-brain barrier (BBB) and neuronal damages. Thus, 8 h immobilization stress, a model of depression in rats leads to leakage of albumin in 14 brain areas that persisted even 24 h after withdrawal of the stress stimuli. This suggests that stress could induce long-term changes in the brain by breakdown of the BBB. There are evidences that long-term stress or stressful life in humans is somehow related to the development of Alzheimer's disease (AD) in their old ages as compared to their stress free counterparts, a possibility arises that breakdown of the BBB in stress could play critical roles. Thus, in present investigation we wanted to know whether AD induced brain pathology caused by amyloid beta peptide (AβP) infusion is exacerbated when the peptide is infused in rats after repeated immobilization stress enough to open the BBB to large molecules e.g., proetins. For this purpose, AβP (1-40) was administered intraventricularly (i.c.v.) in the left lateral ventricle (250 ng/10 µl) of rats (250-300 g body weight) once daily for 4 weeks in naïve animals as well as in rats that were subjected to repeated immobilization stress 2 h daily for 1 week. Control rats received identical dose of saline instead of AβP. In these control and

AβP infused rats BBB breakdown, edema formation, neuronal, glial injuries and AβP deposits in the brain was examined in a blinded fashion by at least 3 independent workers. Our observations showed that repeated 2 h stress for 1 week induced marked BBB breakdown to Evans blue albumin and radioiodine tracers in the cerebrla cortex (55 to 78 %), hippocampus (89 to 98 %), thalamus (34 to 46 %), hypothalamus (35 to 50 %), caudate nucleus (78 to 97 &), cerebellum (80 to 134 %) and brainstem (23 to 35 %) from the naive rats. Infusion of AβP in these stressed rats further enhanced the BBB breakdown to protein tracers by 3- to 5-fold and resulted in pronounced neuronal damages (+400 %), astrocytic activation (+350 %) and brain swelling (+10 %) compared to identical AβP infusion in naive rats. The number of AβP positive cells increased by 3- to 6-fold in these brain areas in stressed group as compared to naive rats. Co administration of PLGA nanoparticles (NPs) loaded cerebrolysin (2.5 ml/kg, i.v. /day from 2nd week of AβP infusion for 2 weeks) induced profound neuroprotection in stressed rats, whereas 5 ml normal cerebrolysin is needed to attenuate brain pathology after AβP infusion in these rats. Taken together our observations are the first to demonstrate that repeated stress exacerbates AD pathology in brain and nanodelivery of cerebrolysin has superior neuroprotective effects in AD.

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Poster

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The University Grants Commission, New Delhi, India (HSS/AS)

Title: Blood-brain barrier disruption, brain edema formation and neuronal and glial injuries caused by Systemic administration of functionalized Gold Nanoparticles

Authors: *P. K. MENON[1], A. SHARMA[2], D. F. MURESANU[3], J. V. LAFUENTE[4], R. PATNAIK[5], A. NOZARI[6], H. S. SHARMA[2];
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Abstract: Gold nanoparticles (AuNPs) are now being used to delivery of drugs to treat Alzheimer's disease (AD), Parkinson's Disease, Stroke and other neurological disorders. Also AuNPs are used for therapeutic or diagnostic purposes in various diseases in clinical medicine. However, the neurotoxic effects of AuNPs are still not well known. Thus, it would b interesting to examine AuNPs neurotoxicity *in vivo* models and to study size related effects of AuNPs on brain pathology. In this investigation we examined the effects of moderate doses of AuNPs of various sizes (5 nm, 10 nm and 40 nm) administered though either intraperitoneally (10 mg/kg, i.p.), intravenously (5 mg/kg, i.v.), intracarotidly (2 mg/kg, i.c.a.) or intracerebroventricularly (20 µg in 20 µl, i.c.v.) in young healthy adult rats (Age 20 to 25 weeks). In these animals blood-brain barrier (BBB) breakdown to Evans blue albumin (EBA 3 ml/kg, i.v.) and [131]-Iodine (100 µCi/kg, i.v.) was examined 24 h after AuNPs administration in the cerebral cortex, hippocampus, thalamus, hypothalamus, cerebellum and brainstem. In these brain regions edema was measured and neuronal injury was examined by Nissl or H&E staining using histopathological techniques. The astrocytic activation was evaluated using immunohistochemistry of glial fibrillary acidic protein (GFAP). Our observations showed marked BBB breakdown (80 to 150 %) in all the above brain areas after administration of 5 to 10 nm AuNPs irrespective of the routs of administration after 24 h as compared to saline treated control group. AuNPs i.p. administration showed a mild but significant increase in BBB breakdown as compared to i.v. administration. Intracarotid administration exhibited more profound leakage of these tracers where I the injected side of the brain whereas i.c.v. administration showed leakage of tracers on the surface of the dorsal side of the brain that was most marked. The other brain areas were less intense in BBB breakdown. Brain edema and neuronal injuries were tightly correlated with BBB breakdown of EBA. Activation of astrocytes was also evident in the areas of neuronal damage and BBB leakage. Interestingly, larger sized AuNPs (40 nm) was not that effective in BBB breakdown either given through i.p., i.v., and i.c.a. or i.c.v. routes. In this group neuronal injuries and brain edema formation was also not much evident. These observations are the first to show that AuNPs induced neurotoxicity is inversely related the size. Smaller AuNPs may have most marked neurotoxic effects than the larger size of AuNPs. Whether co-administration of neuroprotective

drugs e.g., cerebrolysin could attenuate AuNPs neurotoxicity, a feature require further investigation.

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Poster

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UFI 11/32 (JVL); University of Basque Country, Spain

Title: Cerebrolysin reduces exacerbation of nitric oxide synthase, hemeoxygenase and associated blood-brain barrier breakdown and neuropathology following heat stroke in diabetes

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Abstract: Nitric oxide (NO) and carbon monoxide (CO) are free radical gases. Central nervous system (CNS) contains their synthesizing enzymes within the neurons, glial and endothelial cells in forms of nitric oxide synthase (NOS) and hemeoxygenase (HO) abundantly. When these enzymes are activated by trauma, ischemia or CNS insults their activation results in formation of NO and CO from NOS and HO, respectively. Previous studies from our laboratory sowed that a focal spinal cord injury (SCI) resulted in massive expression of NOS and HO in the cord that is positively related with breakdown of the blood-brain barrier (BBB), edema formation and neuronal injuries. However, when brain derived neurotrophic factor (BDNF) or glial derived neurotrophic factor (GDNF) were given topically after SCI, these growth factors are able to thwart NOS and HO production in the cord leading to neuroprotection. This suggests that growth factors may attenuate production of these free radical gases after trauma resulting in neuroprotection. Interestingly, upregulation of NOS and HO is also seen following whole body hyperthermia (WBH) at 38° for 4 h that induces clinical symptoms of brain pathology and heat stroke (HS). When diabetic rats were subjected to identical WBH the stoke symptoms and brain pathology is exacerbated. However, upregulation of NOS and HO in diabetic rats after WBH is not well known. Thus, a possibility exists the diabetic rats after WBH may induce greater overexpression of NOS and HO and that could also be reduced by growth factors treatment. Since cerebrolysin is a balanced composition of several neurotrophic factors and active peptide fragments that reduces WBH pathology in normal and diabetic rats, in this investigation we examined the influence of nanodelivered cerebrolysin on NOS and HO upregulation in diabetic rats after WBH in relation to brain pathology in our rat model. Our observations showed that WBH in diabetic rats resulted in 90 to 130 % overexpression of neuronal NOS (nNOS) and constitutive isoform of HO (HO-2) in the cerebrla cortex, hippocampus, caudate nucleus, thalamus, hypothalamus, cerebellum and brain stem than normal animals. In these animals BBB breakdown, brain edema and neuronal injuries were also exacerbated by 50 to 140 %. Treatment with TiO2 nanodelivered cerebrolysin (2.5 ml/kg, i.v.) or normal cerebrolysin (10 ml/kg, i.v.) 1 or 2 h after a 4 h WBH session resulted in significant neuroprotection and reduction in NOS and HO expression in these brain areas. These observations are the first to show that cerebrolysin is capable to attenuate HO and NOS production in heat stroke resulting in neuroprotection, not reported earlier.

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Poster

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Ministry of Science & Technology, Govt. of India & Govt. of Sweden (HSS/AS)

IT 794/13 (JVL), Government of Basque Country

Title: Nicotine neurotoxicity in cold environment is exacerbated by alcohol and carbon nanoparticles

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Abstract: We have shown previously that nicotine exposure for 1 week significantly increases blood-brain barrier (BBB) breakdown to proetins and induces brain edema and neuronal damages in adult rats. Since nicotine consumed by smoking is often associated with tar inhalation, it is interesting to examine the effects of carbon nanoparticles (NPs) on brain damage induced by nicotine in our model. Furthermore, nicotine consumption is also associated with alcohol intake in the society we also examined a combined effects of alcohol and nicotine on brain pathology. Our previous works showed that nicotine exposure in cold exacerbated brain pathology. Thus, in present investigation, the additional effects of carbon NPs or alcohol in combination with nicotine was also evaluated in animals exposed at cold environment. Rats were treated with nicotine (9 mg/kg/day, s.c.) at room temperature (21±1°C) or at cold environment (8±1°C in a Biological oxygen demand incubator, BOD) for 1 week. In separate group of animals exposed at 21±1°C or 8±1°C, co-administration nicotine and Ethyl alcohol (EtOH, 1.5 g/kg, i.p./day) or carbon NPs (single wall carbon nanotube SWCNT, 10 mg/kg/day, i.p.) was done for 1 week. On the 8th day the BBB permeability to protein tracers (Evans blue albumin, EBA 3 ml/kg, i.v. and [131]-I-Na 100 μCi/kg, i.v.), brain edema (water content) and brain

pathology (neuronal damage using Nissl or H&E on paraffin sections) were evaluated in all the groups in a blinded fashion. Our results show that a combination of nicotine and alcohol exacerbated BBB leakage (EBA by 240 % and radioiodine 300 %), brain edema (water content +1.5 %, volume swelling % *f* 6%) and neuronal injury in the cerebral cortex (120 %) and hippocampus (190%) from nicotine alone at room temperature. Whereas, these changes on BBB, brain edema and neuronal damage were further aggravated by 80 to 120 % at cold environment. Interestingly, the combination of nicotine and SWCNT at cold environment exacerbated brain pathology by 240 to 300 %. On the other hand, only 50 to 90% increase in brain pathology, BBB and edema formation was seen in these rats at room temperature as compared to nicotine alone. These observations are the first to show that the neurotoxicity of nicotine with alcohol combined is exacerbated in cold environment. Furthermore, neurotoxicity caused by nicotine and tar combination is more pronounced at cold environment than nicotine and alcohol combined. It remains to be seen whether a combination of nicotine, alcohol and tar could have further additive brain damaging effects, a feature that is currently being investigated in our laboratory.

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Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

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2011CB50000

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Title: Regulation of mirna biogenesis in neuronal stress

Authors: *Q. YANG[1], W. LI[2], H. SHE[2], J. DOU[2], D. DUONG[3], Y. DU[5], S. YANG[6], N. SEYFRIED[4], Z. MAO[2];
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Abstract: MicroRNAs (miRNAs), small noncoding RNAs, regulate the expression of more than 60% genes and thereby control many cellular processes. The canonical miRNA biogenesis pathway begins in the nucleus with the cleavage of the primary transcripts by the nuclear RNAase III enzyme Drosha. Emerging evidence suggests that the miRNA biogenic cascade may be tightly controlled. Our data show that Drosha is targeted by multiple stress signals. Under stress, p38 MAPK directly phosphorylates Drosha at its N-terminus. This reduces its interaction with DiGeorge syndrome critical region 8, promotes its nuclear export, and facilitates its degradation by calpain. This regulatory mechanism mediates stress-induced inhibition of Drosha. Reduction of Drosha activity sensitizes cells to stress and increases cellular death. Increase in Drosha attenuates stress-induced death. We further confirmed the regulation of Drosha plays a key role in neuronal stress. These findings reveal a critical regulatory mechanism by which stress engages p38 MAPK pathway to destabilize Drosha and thereby inhibit Drosha-mediated neuronal survival.

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Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

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Title: Regulation of nuclear microRNA processing by MeCP2 and impact on dendritic growth

Authors: *T.-L. CHENG, Z. QIU;
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Abstract: Methyl-CpG binding protein 2 (MeCP2) is encoded by X-linked gene MECP2 and its loss- and gain-of-function mutation lead to severe neurodevelopmental disorders such as Rett syndrome and Autism spectrum disorders. MeCP2 is initially recognized as a transcriptional repressor as it recruits HDAC (Histone deacetylase)-mSin3A corepressor complex to methylated DNA sites. It has also been reported that MeCP2 could modulate gene expression post-transcriptionally by regulating microRNA expression. Here we showed that in addition to transcriptional regulation, MeCP2 could suppress nuclear microRNA processing via binding to DiGeorge syndrome critical region 8(DGCR8) directly, a critical component of the nuclear microRNA processing machinery, leading to the disrupted assembly of Drosha/DGCR8 complex. Further analysis showed that MeCP2 C-terminus is the key region for its interaction with DGCR8 but not its DNA binding domain. Protein targets of MeCP2-suppressed microRNAs include CREB, LIMK1 and Pumilio2, which are key factors of neural development. And neuronal morphogenesis such as dendritic development is impaired significantly by autism-associated gain-of-function mutations of MeCP2 and such impairment relies on the interaction of MeCP2 and DGCR8. Thus, control of microRNA processing via direct interaction with DGCR8 represents a novel mechanism for MeCP2 regulation of gene expression and neural development.

Disclosures: **T. Cheng:** None. **Z. Qiu:** None.

Poster

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Title: Loss of Drosha underlies Abeta-induced neurotoxicity

Authors: *Z. MAO[1], W. LI[1], H. SHE[1], J. DOU[1], R. COHEN[2], G. GAO[3], Q. YANG[3];
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Abstract: MiRNAs (microRNAs) are a recently discovered class of non-coding small RNAs that are involved in regulating many cellular processes including stress. Its biogenesis is controlled by several tightly coupled sequential steps initiated in the nucleus by the conversion of the long primary miRNA transcripts to the precursor miRNA by the RNase III enzyme Drosha and subjected to complex regulation. Stress conditions and miRNAs are highly intertwined at several levels. Multiple lines of evidence indicate that miRNAs are especially important to the brain function, modulate pathways and key genes relevant to genetic and sporadic AD, and are themselves altered in AD. Furthermore, inhibition of miRNA biogenesis by deleting Dicer causes progressive neurodegeneration and AD-like tau hyperphosphorylation. However, how these findings translate into animal AD models and human disease remains to be tested. Recently, we have revealed a novel mechanism by which stress induces a p38 MAPK dependent phosphorylation and inhibition of Drosha and triggers cell death. Our unpublished preliminary studies indicate that neuronal stress engages this pathway. Various toxic signals activate p38 MAPK in neurons and induces a p38 MAPK dependent phosphorylation of Drosha. This results in disruption of the microprocessor complex in the neuronal nucleus, leading to Drosha export to the cytoplasm and its degradation. Aβ engages this pathway to trigger dysregulation of Drosha in model specimens. Together, these findings suggest that loss of Drosha may underlie in part Aβ toxicity and possibly the neurodegenerative process in AD.

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Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

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

Title: A sarin-like organophosphate induces non-cholinergic cell death via an inflammatory response in SK-N-SH cells

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Abstract: Introduction Organophosphorus compounds such as sarin are known to irreversibly inhibit acetylcholinesterase (AChE). Recent studies have suggested that these compounds can

induce non-cholinergic toxicity *in vivo* and *in vitro* and that this toxicity involves an inflammatory response. However, the precise mechanisms underlying the cytotoxic effect of organophosphorus compounds are not known. In this study, we sought to elucidate these mechanisms. To this end, we cultured human neuroblastoma cells (SK-N-SH cells) with bis(isopropyl methyl)phosphate (BIMP), a sarin-like organophosphate, and examined the cytotoxicity of BIMP as well as the involvement of the inflammatory response in BIMP-induced neuronal death. Methods In Japan, synthesis and use of sarin is regulated by law. Therefore, we synthesized BIMP, which acts as an AChE antagonist, similar to sarin. We examined the non-cholinergic toxicity of BIMP in SK-N-SH cells. SK-N-SH cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotics at 37°C in a humidified atmosphere with 5% CO2. The cells were exposed to BIMP or vehicle at various concentrations under serum-deprived conditions (0.1% FBS). Cell viability and cytotoxicity were measured using MTT and LDH assay kits, respectively. Cellular morphology was examined by light microscopy, and nuclear morphology was observed by fluorescence microscopy after staining the cells with Hoechst 33342. Prostaglandin E2 (PGE2) content in the medium was measured using an ELISA kit. Total protein was extracted, and western blot analysis was performed to determine cleaved caspase 3, cleaved caspase 9, cleaved PARP, cyclooxygenase 1 (COX-1), and COX-2 expression levels. Results BIMP significantly reduced cell viability and increased cytotoxicity in SK-N-SH cells in a dose-dependent manner. BIMP (500 μM) treatment for 12 or 24 h caused changes in cellular morphology, increased the numbers of floating cells, and resulted in nuclear fragmentation. BIMP significantly increased cleaved caspase 3, caspase 9, and PARP expression in a concentration-dependent manner. Moreover, it induced COX-2 expression and increased PGE2 level. Finally, we examined the effect of a COX-2 inhibitor, ibuprofen, on BIMP-induced cytotoxicity in SK-N-SH cells, and found that ibuprofen pretreatment significantly attenuated BIMP-induced cell death and suppressed COX-2 expression. Conclusion BIMP induces caspase-dependent apoptotic cell death and inflammatory responses in SK-N-SH cells and that the anti-inflammatory drug ibuprofen may protect against BIMP-induced cytotoxicity.

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Poster

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

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Title: Regulation of the expression of neurodegenerative markers through neuronal energy metabolism

Authors: *M. FLORES LEÓN, C. ARIAS ÁLVAREZ;
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Abstract: Introduction: Metabolic conditions associated with the consumption of high fat diets are considered as risk factors for neurodegenerative diseases, for example Alzheimer's Disease, particularly due to the high content of saturated fatty acids such as palmitic acid (PA). It is presumed that PA might be altering the cellular metabolism by modulating the NAD+/NADH relation. Furthermore, a group of Histone Desacetylaces (Class III - Sirtuins) might be modulated by cellular metabolism due to the utilization of NAD+ as a cofactor. Sirtuin 1 (SIRT1) is responsible for the deacetylation of several transcription factors and residues in histone tails, leading to activation or repression of genes. Nevertheless, the mechanisms that relate the intake of high fat diets, the alteration of cellular metabolism and regulation of the expression of neurodegenerative markers, such as the aberrant processing of the amyloid precursor protein (APP) remains to be elucidated. Objective: Analyze the relationship between the inhibition of SIRT1 with the expression of BACE1 and ADAM10 that may lead to an aberrant processing of APP. Metholodogy: Primary hippocampal neuronal cultures in 8 or 12 DIV were exposed to different concentrations of PA or EX527 (SIRT1 inhibitor); 24 hours later protein and RNA were extracted for Western Blot and RT-qPCR analyses. Male Sprague-Dawley rats were intrahippocampally injected with 1 ug/uL of EX527 and 24 hours later they were sacrificed and protein and RNA were extracted for Western Blot and RT-qPCR analyses. Results: Neuronal metabolism was altered by PA resulting in NAD+ depletion and in a reduction of mitochondrial activity. Furthermore, several cytoplasmatic targets of Sirt1 showed changes in their acetylated residues after Sirt1 was inhibited. Conclusions: The alteration of cellular energy metabolism might be implicated in the regulation of the expression of genes implicated in the progression of neurodenerative markers.

Disclosures: M. Flores León: None. **C. Arias Álvarez:** None.

Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 691.06/L12

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

Support: Rao KS received SNI grant from SENACYT

Title: Amyloid induced MRI changes in aged rabbit resembles Alzheimer's disease brain

Authors: K. SAI KRISHNA[1], B. RAMESH[3], M. SHARMA[2], *J. KOSAGISHARAF[4]; [1]Dept. of Med., KIMS Med. Col. and Hosp., Amalapuram – 533 201, India; [2]Dept. of Medicine,, KIMS Med. Col. and Hosp., Amalapuram – 533 201,, India; [3]Dept. of Agr. Biotech., Agr. college, Hassan-573223, India; [4]Ctr. for Neurosci., INDICASAT AIP, Panama City, Panama

Abstract: Alzheimer's disease is most common form of dementia and it is structurally characterized by changes in the brain atrophy and loss of brain volume. The Aβ is one of the most important cause of AD and known to initiate early cascade in AD. Aβ deposition is positively correlated with brain atrophy in AD. Structural brain imaging techniques such as Magnetic Resonance Imaging (MRI) are used to measure neuro-anatomical alterations in Alzheimer's disease brain. Magnetic Resonance Imaging is a non-invasive method to study the brain structure. Objective of the present study is to elucidate the role of Aβ on brain structure of aged rabbit brain. Among 20 aged rabbits, one batch (10) rabbits are injected with Aβ(1-42) and another batch (10) of aged rabbits are injected with saline. The MRI is conducted before Aβ(1-42)/ saline injection and after 45 days of Aβ(1-42)/ saline injection. All the aged rabbits are subjected for MRI and euthanized after 45 days. The MRI results showed a significant reduction in thickness of frontal lobe, hippocampus, midbrain, temporal lobe and increase in the lateral ventricle volume. We also conducted MRI study on 10 AD and 10 normal cases and analyzed for the thicknesses of frontal lobe, hippocampus, midbrain, temporal lobe and lateral ventricle lobe. We found significant reduction in thickness of the frontal lobe and the hippocampus. However, no significant reduction in the thickness of midbrain, temporal lobe and no significant increase in the lateral ventricle volume are observed compared to normal. The results of brain atrophy changes between rabbit brain and human AD brain correlation is established for frontal lobe and hippocampus regions. While, other regions such as midbrain, temporal lobe, lateral ventricle does not show correlation to rabbit brain atrophy changes in the corresponding regions of rabbit brain. The relevance of these changes in AD will be discussed.

Disclosures: K. Sai Krishna: None. **B. Ramesh:** None. **M. Sharma:** None. **J. Kosagisharaf:** None.

Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 691.07/L13

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

Title: Safeguarding effect of protein disulfide isomerase in Aβ intra-hippocampal injected rats; time matters

Authors: *P. SADEGHI[1,2], F. SHAERZADEH[1,2,3], F. KHODAGHOLI[1,2];
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Abstract: Introduction Disulfide bonds, covalent cross-link between cysteine residues of proteins, are often formed to stabilize protein structures or join the other proteins. Improper interaction between cysteine residues results in misfolded proteins. To avoid this mismatch, protein folding in endoplasmic reticulum (ER) is assisted by an elaborate system of chaperones and catalysts. Protein disulfide isomerase (PDI), a retained molecule in ER, acts as a molecular chaperone and folding catalyst by introducing disulfide bonds into protein structures and further catalyzing the rearrangement of incorrect disulfide bonds. Also, in spite of the physiological concentrations of nitric oxide (NO) that mediates normal signaling, excessive generation of NO by overactivation of NO synthase results in neurotoxicity and neuronal injury. Methods Adult male Wistar rats were divided into two groups. First group received the intra-hippocampal injection of 3 µl PBS bilaterally and were decapitated immediately after injection. Second group were injected by 3 µl Aβ1-42 (10 ng/µl) in CA1 region of hippocampus and next the animals were killed 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days after Aβ injection. Reductase activity of PDI was assayed by measuring enzyme-catalyzed reduction of insulin in the presence of dithiothreitol (DTT). To measure nitrites content, homogenates were prepared in Tris-HCl (20 mM, pH 7.3, 1:10) and the absorbance was measured at wavelength of 550 nm. Results We tried to determine the role and trend of ER specific molecules as PDI in neural defense system beside NO determination in intra-hippocampal amyloid beta (Aβ)-injected rats in 10 days running. PDI is considered as a member of the thioredoxin superfamily. Based on the specific reaction that catalyzes, PDI is known as a critical molecule involved in proper folding of protein structures in ER. Two days after injection of Aβ, PDI activity significantly decreased compared to control group. This trend of decline continued till 10th day after Aβ injection. From the other side, one day after Aβ injection, concentration of NO started to increase and reached to the maximum level 6 days after injection of Aβ. Afterward, level of NO decreased but the level of NO in 10th day was still higher than the control group. Correlations and regression between NO level and PDI activity were significant ($P < 0.001$) in Aβ-injected rats. There is a negative correlation between these two factors with a good regression line fit ($R2=0.73$) indicating that increase in NO level was concomitant with decrease in PDI activity.

Disclosures: **P. Sadeghi:** None. **F. Shaerzadeh:** None. **F. Khodagholi:** None.

Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

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

Support: Childhood Cancer Foundation (PROJ13/027)

Cancer Foundation (CAN 2014/707)

Swedish governmental grants to scientists working in health care (ALFGBG-429271)

Title: Radiation induces death of proliferating cells, microglia activation, and blood brain barrier damage in the cerebellum after cranial irradiation in young rats

Authors: ***K. ZHOU**[1], J. EK[1], M. BOSTRÖM[1], T. LI[1], C. XIE[1], Y. XU[1], K. BLOMGREN[2], C. ZHU[1];
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Abstract: Background: Posterior fossa tumors are the most common childhood intracranial tumors, and radiotherapy is one of the most effective tools for treating children with such tumors. However, radiotherapy has both acute and long-lasting adverse effects, and neurocognitive impairments have significant impacts on the patient's quality of life and are the most important measurements of brain tumor therapy outcomes in clinical trials second only to survival. **Purpose:** The purpose of this study was to characterize the mechanisms of irradiation-induced cerebellum injury. **Methods**: Male Wistar rats were subjected to a single whole-brain dose of 6 Gy irradiation on postnatal day 11. The animals were sacrificed at 6 h or 24 h after irradiation to detect cell death, microglia activation, blood brain barrier permeability, and cell proliferation in the cerebellum or at 16 weeks after irradiation to evaluate cerebellum injury. **Results:** An increasing number of pyknotic cells were detected in the external granular layer (EGL) of the cerebellum at 6 h and at 24 h after irradiation as indicated by H&E staining. This was further confirmed by active caspase-3 staining. The number of replicating cells in the EGL as indicated by BrdU labeling and Ki67 immunostaining was decreased at 6 h after irradiation and much more dramatically at 24 h after irradiation. Microglia invasion and activation in the EGL increased after irradiation as indicated by Iba-1 and CD68 staining. The blood brain barrier permeability increased after irradiation as indicated by albumin staining. The volume of the EGL

increased by about 25% at 6 h and then decreased by about 50% at 24 h after irradiation. Behavior tests showed that irradiated rats spent more time in the open zone area in the open field test and that recognition function was impaired. The total volume of the cerebellum was reduced by almost 50% at 16 weeks after irradiation; however, the total number of Purkinje cells was not significantly different after irradiation compared with controls. **Conclusions:** The cerebellum is more sensitive to irradiation-induced injury, including the death of proliferating cells, microglia activation, and blood brain barrier damage, and it undergoes persistent cerebellar atrophy. All of these factors are probably related to neurocognitive impairment.

Disclosures: **K. Zhou:** None. **J. Ek:** None. **M. Boström:** None. **T. Li:** None. **C. Xie:** None. **Y. Xu:** None. **K. Blomgren:** None. **C. Zhu:** None.

Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

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Program#/Poster#: 691.09/L15

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

Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)

Title: Palmitate impairs insulin signaling in hippocampal neurons

Authors: *H. M. DE MELO[1], G. S. SEIXAS DA SILVA[2], S. T. FERREIRA[2], F. G. DE FELICE[2];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to memory loss and cognitive impairment. The causes for development of sporadic AD, which is markedly associated with aging, are still largely unknown. AD brains present Abeta accumulation, tau hyperphosphorylation and impaired insulin signaling, which are thought to mediate synapse and cognitive defects. Indeed, patients with diabetes and obesity have increased risks of developing AD and some studies have supported the emerging idea that AD is a novel brain-specific form of diabetes. Lipotoxicity is a key mechanism in the development of peripheral insulin resistance in obese and type 2 diabetes patients, known to have increased

circulation of saturated fatty acids (SFA). This study aimed to evaluate whether palmitate, the most abundant circulating SFA, inhibits insulin signaling and cause AD markers in cultured hippocampal neurons. Hippocampal neurons were exposed to palmitate and levels of IRS-1 serine phosphorylation (Ser312, Ser307 and Ser636), known to mediate insulin resistance, were analyzed by immunocytochemistry. Results show increased IRS-1 serine phosphorylation and tau phosphorylation in palmitate-exposed neurons as compared to vehicle, similar to what is observed in Abeta-exposed hippocampal neurons. Our findings indicate that palmitate impairs neuronal insulin signaling by inhibiting IRS-1 signaling and causes tau phosphorylation. Thus, common mechanisms accounting for peripheral insulin resistance, including SFA signaling, may be at play across the blood-brain barrier, instigating the initial steps of AD and likely explaining why peripheral metabolism impairments represent a risk factor for AD development.

Disclosures: H.M. De Melo: None. **G.S. Seixas da Silva:** None. **S.T. Ferreira:** None. **F.G. De Felice:** None.

Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 691.10/L16

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

Title: Manganese loading induces male interspecific predatory aggression in nonaggressive rats

Authors: *L. GELAZONIA, I. LAZRISHVILI, T. BIKASHVILI, N. MITAGVARIA;
I. Beritashvili Ctr. Of Exptl. Biomedicine, Tbilisi, Georgia

Abstract: Generally accepted that aggression and violent behavior may stem from toxic metals due to their excessive accumulation in the body. Despite of numerous studies on the problem of neurotoxicity of manganese, issues concerning its role in aggressive and violent behavior manifestations are almost unknown. The published data concerning this problem are indirect and often - contradictory. The effect of 30 (group I) and 75 (group II) day exposure to different doses of manganese chloride - MnCl2.4H2O (10 and 15 mg/ml in drinking water) on interspecific predatory aggression (IPA) of the initially nonaggressive rats was studied. Prior to the MnCl2.4H2O loading, to eliminate any spontaneously aggressive animals from subsequent experiments, the rats were tested for male interspecific predatory aggression. 30-day exposure to 10mg/ml MnCl2.4H2O does not stimulate aggressive behavior of rats, while after exposure to the higher dose 37,5% of rats manifested IPA and 25% attempted IPA. 75-day exposure to 10mg/ml MnCl2.4H2O 37,5% of rats manifested IPA and 37,5% - IPA attempt. After exposure

to the higher dose 75% of rats manifested IPA attempt and 25% - nonaggressive behavior. The data obtained support the idea that excess of Mn in the body might cause of rats' aggressive behavior. As we see, the 30-days loading by Manganese Chloride (10 mg/ml) is not sufficient for creation of that cumulative dose, which is needed for revealing of aggressive behavior. It is know that long-term high dose Mn-intoxication leads to an abrupt disruption of neurotransmitter systems, especially nigrostriatal dopaminergic system. According to our assumption this violates the motor activity and animals despite repeated attempts are not able provide IPA.

Disclosures: **L. Gelazonia:** None. **I. Lazrishvili:** None. **T. Bikashvili:** None. **N. Mitagvaria:** None.

Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 691.11/L17

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

Support: CONACyT INFR-251132

CONACyT RT-224359

Title: Hexahydrobenzene produces oxidative stress, and glial reactivity in the adult mouse hippocampus

Authors: ***T. V. CAMPOS ORDONEZ**[1], D. ZARATE-LOPEZ[1], V. LOPEZ-VIRGEN[1,2], J. VEGA-RIQUER[1,2], A. GALVEZ-CONTRERAS[3], N. MOY-LOPEZ[1], J. GUZMAN-MUNIZ[1], O. GONZALEZ-PEREZ[1];
[1]Sch. of Psychology/ Lab. of Neurosci., Univ. of Colima, Colima, Mexico; [2]Med. Sci. PhD Program, Sch. of Medicine, Univ. of Colima, Colima, Mexico; [3]Dept. of Neurosci., Ctr. Universitario de Ciencias de la Salud, Univ. of Guadalajara, Guadalajara, Mexico

Abstract: Background: Volatile compounds are the fourth most commonly drug of abuse after alcohol, tobacco, and marijuana. Hexahydrobenzene, also referred as Cyclohexane, is a volatile solvent used as a substitute of dangerous organic solvents in several products, such as: paints, paint thinners, lacquers, gasoline, adhesives, etc. Previous studies have found cellular and behavioral disturbances after inhaling organic solvents. Unfortunately, there is little investigation about the effects of cyclohexane in the Central Nervous System (CNS). Objective: To analyze the effects of cyclohexane inhalation on motor behavior, spatial memory, and reactive gliosis

CA1 and CA3 regions in the hippocampus of adult mice. Method: We use in male Balb/C mice (P60) divided in two groups: controls and the cyclohexane group (exposed to 9,000 ppm cyclohexane daily for 30 days). We evaluated motor skills with a functional observational battery (FOB) and memory performance with the Morris water maze (MWM). We analyzed the expression of AP endonuclease 1 (APE1), astrocytes (GFAP cells +) and microglia (Iba1+ cells) in the hippocampal CA1 and CA3 regions. Statistical analysis was done with the Mann-Whitney ''U'' test. Results: The cyclohexane group showed statistically significant differences in gait, activity function, sensory auditory function, response to two-hand manipulation, forelimb/hindlimb grip strength, salivation and lacrimation as compared to the control group. We did not find statistically significant differences in memory performance in the MWM. Interestingly, we found a significantly increased in the expression of APE1 in the cyclohexane group in the CA1 region (IQR = 1.3-1.6; P = 0.04) and in the CA3 (IQR = 1.7-2.1; P = 0.03) as compared to controls. In the CA1 area the number of astrocytes in the cyclohexane group (IQR = 29.6-35.3) was higher than in the control group (IQR = 25.5-32.6). In the CA3 area, cyclohexane increases significantly in the number of astrocytes (IQR = 37-45.4) as compared to the control group (IQR = 22.9-29.8). The number of microglial cells was increased in the CA1 region of the cyclohexane group (IQR = 11.8-12.3) as compared to controls (IQR = 8.5-9.2). In the CA3 region, the cyclohexane group also showed an increase (IQR = 5-7.9) in the number of microglia cells as compared to the control group (IQR = 2.7-2.9). Conclusion: Cyclohexane produces functional deficit without changing learning and spatial memory. Furthermore, cyclohexane induces oxidative stress, astrogliosis and microglial reactivity in the hippocampal CA1 and CA3.

Disclosures: **T.V. Campos Ordonez:** None. **D. Zarate-Lopez:** None. **V. Lopez-Virgen:** None. **J. Vega-Riquer:** None. **A. Galvez-Contreras:** None. **N. Moy-Lopez:** None. **J. Guzman-Muniz:** None. **O. Gonzalez-Perez:** None.

Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 691.12/L18

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

Support: NIH grant NS076448

Title: Intranasal brain delivery of obidoxime prevents mortality and cns damage from organophosphate poisoning

Authors: *J. KRISHNAN[1], P. ARUN[1], A. APPU[1], N. VIJAYAKUMAR[1], T. FIGUEIREDO[1], M. BRAGA[1], S. BASKOTA[1], 1. FREY, II[2], J. MOFFETT[1], A. NAMBOODIRI[1];
[1]APG, USUHS, Bethesda, MD; [2]Ctr. for Memory & Aging, Regions Hospital, Hlth. Partners Inst. for Educ. and Res., St. Paul, MN

Abstract: Intranasal delivery is an emerging method for bypassing the blood brain barrier (BBB) and targeting therapeutics to the central nervous system (CNS). This method has been shown in numerous studies to bypass the BBB and rapidly deliver drugs to the CNS via extracellular and paracellular routes along the olfactory and trigeminal neural pathways. This approach is particularly advantageous because charged molecules and high molecular weight compounds, which cannot penetrate the BBB, can be rapidly delivered to the CNS in therapeutic doses. Oximes are used to counteract the effects of organophosphate poisoning, but they do not readily cross the BBB. Therefore, they cannot effectively counteract the central neuropathologies caused by cholinergic over-activation when administered peripherally. For these reasons we examined intranasal administration of oximes in an animal model of organophosphate poisoning to determine their effectiveness in reducing mortality and seizure-induced neuronal degeneration. Intranasal application of obidoxime (OBD) was effective in partially reversing paraoxon-induced acetylcholinesterase inhibition in the brain and also reduced seizure severity and duration. Further, intranasal OBD completely prevented mortality and nerve cell loss. Fluoro-Jade-B staining revealed extensive neuronal degeneration in the surviving saline-treated animals 24 hours after paraoxon administration, whereas no detectable degenerating neurons were observed in animals given intranasal OBD 30 min before or 5 min after paraoxon administration. Intranasal administration is an effective method for rapidly bypassing the BBB and noninvasively delivering oximes such as OBD to the CNS to counteract the central neuropathologies caused by organophosphate poisoning. Now that this method has been found effective, other neuroprotective modalities, including organophosphate scavenging enzymes, are potentially promising candidates for use via the intranasal route. NIH grant NS076448.

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Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 691.13/L19

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

Title: The zombie apocalypse: neurodegeneration in Alzheimer's disease includes the apoptosis of healthy bystanders near "undead" senescent neurons

Authors: *H. CHOW[1,2], K.-H. TSE[1], K. HERRUP[1];
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Abstract: Age is by far the greatest risk factor for Alzheimer's disease (AD) and cellular senescence, a factor strongly correlated with aging, would this be expected to play a role in the AD neurodegenerative process? During neuronal stress, elevated intracellular calcium leads to activation of calpain and cleavage of the Cdk5 activator, p35, to p25. Subsequently, p25 binds to GSK3β, blocking Axin binding and inhibiting access of GSK3β to its β-catenin substrate. This results in elevated levels and enhanced nuclear presence of β-catenin. The increased nuclear localization of β-catenin favors its binding to CBP, a transcriptional cofactor, over its alternate partner, p300. CBP/β-catenin association leads to a switch in downstream targets, from p300-related neuronal maintenance genes to CBP-related genes with pro-proliferation properties. Thus, overexpression of p25 in neurons leads to enhanced β-catenin activity and increased expression of target genes such as cyclin D and c-Myc. This correlates with neuronal cell cycle re-entry, but in p25 expressing cells cell cycle activity results not in neoplastic transformation, but in cellular senescence. β-catenin-positive cells express senescence markers such as β-galactosidase and release senescence related cytokines. These secreted factors trigger apoptosis in nearby untransfected bystander neurons. This bystander effect is also seen in mouse models of AD. Although the levels of total β-catenin are reduced, we find patches of neurons in hippocampus and cortex with strong nuclear β-catenin signals and markers of cellular senescence. The soluble Wnt antagonists, DKK1 and sFRP2, are targets of β-catenin and both are released from both p25- and β-catenin-expressing neurons. They are the likely agents of the bystander effect as DKK1 and sFRP function-blocking antibodies prevent it, despite the persistence of senescence markers in the p25-expressing cells. Thus, induction of senescence occurs in neurons that appear to be cycling. The resulting bystander effect suggests that these abortive attempts at cell division mark zombie-like cells in neuronal populations at risk for death in AD; and in AD, as in Hollywood, the victims of this conversion are innocent bystander neurons in the region.

Disclosures: **H. Chow:** None. **K. Tse:** None. **K. Herrup:** None.

Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

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

Support: DTRA grant CBM.THRTOX.01.10.RC.021

NIAID grant AOD12058-0001-0000

Title: Facilitation of neurotransmission in bont/a-paralyzed nerve terminals by 3,4-diaminopyridine does not act through the incorporation of cleaved snap25 in vesicle release

Authors: *A. B. BRADFORD[1], T. M. RUSSO[2], E. J. GLOTFELTY[2], K. M. HOFFMAN[1], P. H. BESKE[1], P. M. MCNUTT[1];
[1]Cell. and Mol. Biol., US Army Med. Res. Inst. of Chem. Def, Gunpowder, MD; [2]Cell. and Mol. Biol., US Army Med. Res. Inst. of Chem. Def., APG-EA, MD

Abstract: Botulinum neurotoxins (BoNTs) are highly lethal Tier-1 Biothreat toxins that cause muscle paralysis by targeting and enzymatically cleaving SNARE proteins in motor neurons, thus preventing synaptic vesicle release. In particular, BoNT serotype A (BoNT/A) is a potent and persistent toxin that remains enzymatically active in motor nerve terminals for months, resulting in a sustained paralysis. There are currently no countermeasures to reverse BoNT-intoxication. 3,4-diaminopyridine (3,4-DAP) is a small molecule that has been found to improve neuromuscular transmission for various myasthenic diseases. Clinical evidence suggests that administration of 3,4-DAP transiently improves skeletal and respiratory muscle response in some cases of BoNT/A poisoning. 3,4-DAP specifically blocks voltage-gated potassium channels in neurons, thereby prolonging action potential duration. It has been hypothesized that prolonged AP firing renders cleaved SNAP-25 (cSNAP-25) capable of supporting acetylcholine (ACh) release from BoNT/A-intoxicated motor nerve terminals, but the specific mechanism by which this occurs is unclear. We tested the validity of this hypothesis in isolated mouse phrenic nerve-hemidiaphragm preparations. Application of 10 μM 3,4-DAP to control preparations increased twitch tension strengths by 200-300% within 15 min. Bath application of 6.7, 67 or 670 pM BoNT/A eliminated nerve-elicited muscle contractions with times-to-paralysis that were dose-dependent. At all BoNT/A doses, increasing degrees of partial paralysis reduced the capacity for 3,4-DAP to increase twitch tensions. Importantly, 3,4-DAP did not restore nerve-elicited contractions when applied to fully paralyzed muscles. These results suggest that 3,4-DAP does not enable neurotransmission via incorporation of cSNAP-25 into the vesicle release machinery. We are continuing to explore the conditions and mechanisms by which 3,4-DAP may be able to restore suprathreshold ACh release. Additional approaches, such as the use of tetanic stimulation and muscle endplate potential recording, are underway to allow more sensitive measurements of the 3,4-DAP effect. Mechanistic understanding of how 3,4-DAP potentiates neurotransmission in BoNT/A-impaired neuromuscular junctions will assist in clinical decision-making as well as development of therapeutic approaches to treat BoNT intoxication.

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Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

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

Support: JSPS KAKENHI 25750207

JSPS KAKENHI 15K01419

Title: High-density cobalt induces axonal degeneration and inhibits the motility of axonal motile mitochondria

Authors: *S. KIKUCHI[1], T. NINOMIYA[1], T. KOHNO[2], T. KOJIMA[2], H. TATSUMI[1]; [1]Dept. of Anat. 1, Sapporo Med. Univ. Sch. of Med., Sapporo, Japan; [2]Dept. of Cell Sci., Res. Inst. for Frontier Medicine, Sapporo Med. Univ., Sapporo, Japan

Abstract: A recent report has demonstrated that some patients with a metal-on-metal (MoM) hip implant suffer from peripheral or optic nerve neuropathies. Cobalt is one of the main components of the implant and the blood cobalt concentration in patients with a MoM hip implant is higher than normal. However, the mechanisms of neuropathy induced by high cobalt have yet to be elucidated. In the present study, we investigated how cobalt may affect the mitochondrial dynamics in primary cultures of rat dorsal root ganglia (DRG). We assessed (1) changes in the number of motile mitochondria before and after cobalt exposure and (2) the number of swellings per 100 μm axonal length. To observe the axonal mitochondria dynamics, mitochondria in the axons were visualized by the transfection of Lentivirus vectors containing mitochondria-targeted DsRed2 sequences. DRG cultures were produced from 19-day-old embryo of Sprague-Dawley rats. At 4 weeks, the cultures were transfected with Mito-DsRed2 and incubated another 2-3 weeks. Time-lapse imaging was used to assess the motile mitochondria in the axons. Both before and after the cobalt treatment, time-lapse imaging was used to assess the motile mitochondria in the axons. Images were taken every 6 seconds in each axon with a total of 200 images produced pre- and post-treatment. The duration of exposure to the cobalt chloride was 24 hours. The cobalt chloride concentrations of the replacement medium were 0, 200, 400, 600 or 800 μM. After taking the time-lapse images, the cultures were fixed with 4% paraformaldehyde and immunohistochemically stained with a neurofilament protein antibody to count the number of the axonal swellings. The rate of the motile mitochondria in the 600 and 800 μM cobalt concentrations significantly decreased to 27.3% and 0%, respectively. Of particular note, the 800 μM cobalt density suppressed the axonal mitochondria transport completely. The number of

swellings totaled 0.2±0.41, 0.1±0.31, 0.55±0.76, 2.1±1.65 and 5.05±1.85 (average ± standard deviation, n=20) in 0, 200, 400, 600 and 800 μM cobalt concentrations, respectively. Significant differences were recognized between the high densities of cobalt (600 and 800 μM) and the low (0, 200 and 400 μM). Since axonal swelling is thought to occur before axonal degeneration, we concluded from our results that the high-density cobalt caused axonal degeneration in the DRG cultures. Although the molecular mechanisms of neurotoxicity induced by cobalt are not clear, our results showed that high cobalt concentrations induced peripheral neural degeneration by inhibiting axonal motile mitochondria and axonal swelling in the peripheral nerves.

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Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 691.16/L22

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

Support: CONACYT 219703

Title: Oxidative stress effect on purinergic receptors and Glycogen synthase kinase 3 beta in hippocampus of rats exposed lo ozone

Authors: ***R. V. PEREZ**, E. CARMONA-MONTESINOS, E. RODRIGUEZ-MARTINEZ, S. L. RIVAS-ARANCIBIA;
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Abstract: Ozone exposure at low dosis causes oxidative stress and induces a progressive neurodegeneration in hippocampus of rats when they are chronically exposed to this gas. Adenosine 5´ triphosphate (ATP) can act as a mediator in intercellular communication between neurons and glial cells. The increase of extracellular ATP is associated with inflammation and celular death processes. On the other hand activation of purinergic receptors induces activation of intracellular signaling cascades in which synthase kinase 3 beta (GSK3 β) could be involved. The objective of this work was to study the effect of chronic low ozone dosis on the P2X7 and P2Y2 receptor expression as well as the activity of GSK3β in rat hippocampus. For this purpose there were used 36 Wistar male rats with free acces to food and water, that were housed individually, and randomly divided in 6 groups, each group received one of the following treatments: group 1: control exposed to air ozone free environment, group 2, 3, 4, 5 and 6

exposed to ozone for 7, 15, 30, 60 and 90 days respectively. The ozone exposure was for 4 hours daily at 0.025 ppm.Two hours after the end of treatment, rats were then deeply anesthetized, and processed for western blot and immunohistochemistry techniques using specific antibodies against P2X7,P2Y2 and GSK3β.Results showed an P2X7 receptor increase expression at 60 days of ozone exposition in comparison to the control group, and also we found an increase in the immunoreactivity. Moreover results showed an P2Y2 increase at 15, 30, 60 and 90 days of ozone exposure with respect to the control, as well as, an increase in the P2Y2 immunoreactivity. Results also showed an GSK3β phosphorylation increase at serine 9 which started from 15 to 90 days of ozone exposure. This results indicate that oxidative stress caused by ozone can alter the purinergic receptors expression, mainly the P2Y2 receptor, and lead to the GSK3β phosphorylation, which has an important biological role in inflammation and celular death during neurodegenerative process. We thank Gabino Borgonio Perez for his technical assistance. This work was supported by CONACYT 219703

Disclosures: R.V. Perez: None. **E. Carmona-Montesinos:** None. **E. Rodriguez-Martinez:** None. **S.L. Rivas-Arancibia:** None.

Poster

691. Oxidative Stress-Induced and Other Mechanisms of Neurodegeneration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 691.17/L23

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: This work was supported by DGAPA IN221114 to S. R-A

Title: Ozone exposition induces apoptotic cell death mediated by the endoplasmic reticulum stress in hippocampus of rats

Authors: *A. E. RODRIGUEZ[1], M. MÉNDEZ ARMENTA[2], C. NAVA-RUÍZ[2], S. L. RIVAS-ARANCIBIA[3];
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Abstract: Endoplasmic reticulum stress caused protein misfolding and the release of calcium, which lead to caspase 12 activation, which contributes to progressive neurodegeneration process. The aim of this work was to study the effect of oxidative stress on the endoplasmic reticulum (ER) and its relation to cell death in the hippocampus of rats exposed to ozone. For this purpose

we carried out the following experiments. Seventy-two male Wistar strain rats were randomly divided into 6 experimental groups, each group received one of the following treatments: Group 1) control was exposed to air without ozone; Group 2, 3, 4, 5 and 6 were exposed to ozone (0.25 ppm) for 7, 15, 30, 60 and 90 days respectively. Two hours after the last exposure to ozone, the animals were deeply anesthetized, the brains were removed and the hippocampi were processed for the following techniques: TUNEL, western blot, and immunohistochemistry, with the following antibodies: ATF6 and GRP78 for studying stress of endoplasmic reticulum, we used caspase 12 and TUNEL to study apoptotic cell death and electronic microscopy for the study of ultraestructural changes of endoplasmic reticulum. The results showed that TUNEL-label cells increased in groups exposed to ozone from 30 until 90 days. An increase was also observed in the immunoreactivity of ATF6 and RP78 from 30 days. In addition the ATF6 and GRP78 expression was significantly increase in groups exposed to ozone for 60 days ($p< 0.05$) and 90 days ($p<0.05$) compared with the control group. However, there was also an increase of caspase 12 at 90 days ($p<0.05$) of exposure to ozone compared to control group. Moreover, we observed dilatation and loss of continuity of the membranes of the endoplasmic reticulum from 30 to 90 days of exposure to ozone. In conclusion, the ozone exposure produces endoplasmic reticulum stress; this condition leads to apoptotic cell death through the caspase 12 activation, like what might occur in Alzheimer's disease.

Disclosures: A.E. Rodriguez: None. **M. Méndez Armenta:** None. **C. Nava-Ruíz:** None. **S.L. Rivas-Arancibia:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.01/L24

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

Support: NIH/NIDA RO1 DA034783

Title: Characterization of chronic methamphetamine neurotoxicity in Parkin knockout rats

Authors: *A. SHARMA, A. FLACK, A. MOSZCZYNSKA;
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Abstract: Methamphetamine (METH) is one of the most widely used addictive substances. METH is a potent sympathomimetic amine, which affects both central and peripheral nervous system. Both binge and chronic METH is highly toxic to dopaminergic (DAergic) and

serotonergic (5HTergic) nerve terminals in the striatum. Neurotoxicity of METH occurs by multiple mechanisms, primarily via DA-mediated oxidative stress and is manifested by persistent decreases in DAergic and 5HTergic markers, including DA, 5-HT, and their metabolites as well as by astrogliosis. We have previously shown that binge METH oxidatively damages Parkin and decreases its levels in rat striatal synaptosomes shortly after the last dose of the drug. We have also shown that overexpression of Parkin protects against neurotoxicity of binge METH. The objective of the present study was to characterize neurotoxicity of chronic METH in wild type (wt) and Parkin knockout (KO) Long Evans rats. We hypothesized that Parkin-deficient rats would be more sensitive to METH toxic effects. To test this hypothesis, adult male Long Evans rats were treated with chronic doses of METH (wt: 20 mg/kg; KO: 10 mg/kg, for 10 days, once a day, i.p) or saline and sacrificed 5 or 10 days after the last injection. High performance liquid chromatography (HPLC) analysis of striata revealed no statistically significant decreases in 5HTergic or DAergic markers after drug administration. When striatal sections from saline- and METH-treated rats were immunolabeled for tyrosine hydroxylase (TH), a DAergic marker, and glial fibrillary acidic protein (GFAP), an astrocyte marker, TH immunofluorescence was decreased (-25%) while GFAP immunofluorescence was increased (5d: 4 fold; 10d: 3 fold) in METH-treated wt rats as compared to wt saline controls. Parkin KO rats showed no statistically significant decreases in striatal DAergic and 5HTergic markers and similar changes in TH and GFAP as wt rats, a 25% decrease and 3 fold increase, respectively, at 10 days after chronic METH. As Parkin KO rats were treated with two times lower doses of METH (due to high mortality rate), our findings suggest that Parkin KO rats are more sensitive to METH toxic effects than wt Long Evans rats. Moreover, our data also suggest that wt Long Evans rats are more resistant to METH neurotoxicity than Sprague Dawley rats. In summary, our study demonstrates reactive astrogliosis in chronic METH-treated Long Evans rats and suggests that loss of parkin protein results in higher response of astrocytes to METH neurotoxicity. Further studies are warranted to establish the factors responsible for species differences in response to chronic METH and role of parkin in METH-induced astrogliosis.

Disclosures: **A. Sharma:** None. **A. Flack:** None. **A. Moszczynska:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

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Program#/Poster#: 692.02/L25

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

Support: DA034783

DA024923

Title: Colonic neurotoxicity in rats that self-administer methamphetamine

Authors: A. FLACK[1], A. L. PERSONS[2], S. KOUSIK[3], *J. M. GEMECHU[1], T. NAPIER[3], A. MOSCZCYNSKA[1];
[1]Postdoctoral fellow, Wayne State Univ., Detroit, MI; [3]Ctr. for Compulsive Behavior and Addiction, [2]Rush Univ., Chicago, IL

Abstract: Methamphetamine (METH) is a highly abused psychostimulant that is associated with an increased risk for developing Parkinson's disease (PD) (Callaghan et al. Drug Alcohol Depend. 120:35, 2012; Curtin et al. Drug Alcohol Depend. 146:30, 2015). This enhanced vulnerability may relate to the known neurotoxic effects of METH that are also observed in PD pathology. High-dose METH (Butler et al. J Addict Prev 2004) increases α-synuclein levels and decreases the E3 ligase parkin in the striatum of rats, and in PD, α-synuclein is increased in the substantia nigra (Recasens and Dehay Neuroanat 2014). Peripheral factors may contribute to CNS pathology. α-Synuclein levels in the enteric nervous system are increased in PD patients (Shannon et al. Mov Disord. 27:709,2012), as well as in rats that self-administered (SA) METH prior to tyrosine hydroxylase (TH) deficits in the striatum (Napier et al. SfN Abstr #240.12, 2013; Kousik et al. Eur J Neurosci.40:2707, 2014). As in PD patients and rats that SA meth, gut α-synuclein pathology precedes brain monoamine pathology, and we hypothesized that a similar pattern would occur with gut monoamines (i.e., dopamine and norepinephrine). Thus, the aim of the present study was to measure the levels of α-synuclein, TH and dopamine-β hydroxylase (DβH), a noradrenergic marker, at different times following withdrawal from self-administered METH in the distal colon of rats. Male Sprague-Dawley rats were allowed to self-administer METH for 3h per day for 14 days (d) while yoked controls received saline. Colon tissue was harvested at 24h, 14d, and 56d after cessation of the operant task. As compared to their saline-yoked counterparts, METH exposed rats showed a trend for an increase in α-synuclein immunoreactivity at 24h that returned to baseline levels by 14d. At 24h, significant deficits in both TH and DβH immunoreactivity were observed; the deficit in DβH persisted at least up to 14d. The monaminergic markers co-localized with neurofilaments. Our results, coupled with those from our previous studies, indicate that the colon demonstrates an early onset neurotoxicity that may predispose human METH users to develop PD. Supported by DA034783, DA024923 and the Center for Compulsive Behavior and Addiction.

Disclosures: A. Flack: None. **A.L. Persons:** None. **S. Kousik:** None. **J.M. Gemechu:** None. **T. Napier:** None. **A. Mosczcynska:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

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Program#/Poster#: 692.03/L26

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

Support: NIH/NIDA R01 DA034783

Title: Alterations in phospho-ser129-α-synuclein in the striatum in rats after binge METH may provide clues to understanding the link between METH abuse and PD

Authors: J. M. GEMECHU, *A. FLACK, A. ALBRECHT, A. MOSZCZYNSKA;
Wayne State Univ., Detroit, MI

Abstract: Methamphetamine (METH) is a psychostimulant drug which widely abused in the US and worldwide. At high doses, it causes neurotoxicity in rats, non-human primates, and humans. Previous studies have shown that exposure to METH in earlier stages of life increases the likelihood of developing Parkinson's disease (PD) later on. PD and METH neurotoxicity share several hallmarks of neurodegeneration. For example, in both conditions, tyrosine hydroxylase (TH), a dopamanergic (DAergic) marker, is decreased while α-synuclein is increased in the striatum. The most frequent modification of α-synuclein in PD patients is its aggregation and phosphorylation at Ser129. Phospho-Ser129-α-synuclein is predominantly localized to striosomes in the striatum with lower levels in the matrix. Differential losses in DAergic markers between striosomes and matrix were observed in rodents. It is not known how neurotoxic doses of METH change the levels of phospho-Ser129-α-synuclein in striatal compartments. The matrix contains higher DAergic inervation than the striosomes. We have hypothesized that METH binge will increase the immunoreactivity of phospho-Ser129-α-synuclein in striatal matrix compared to saline (SAL) treated controls. To test our hypothesis rats were treated with 4x7.5mg/kg METH-HCl, euthanized at 7 d after the binge, and examined for changes in immunoreactivity of phospho-Ser129-α-synuclein in the striatum. Co-immunostaining for TH and phospho-Ser129-α-synuclein revealed an increase in phospho-Ser129-α-synuclein immunoreactivity in TH+ axons in the matrix and striosomes. Phosphorylated α-synuclein appeared aggregated in TH+ axons of the matrix. Previous studies have found that approximately 90% of α-synuclein deposits found in Lewy bodies have been phosphorylated at Ser129. Consequently, our findings suggest that aggregated phospho-Ser129-α-synuclein mediates METH neurotoxicity and potentially also susceptibility of human METH users to develop PD later in life. Further examination of this biomarker in METH neurotoxicity is warranted to provide better understanding of the link between METH abuse and PD. Supported by by NIH/NIDA R01 DA034783. Keywords: Methamphetamine, phospho-Ser129-α-synuclein, tyrosine hydroxylase, striosome

Disclosures: **J.M. Gemechu:** None. **A. Flack:** None. **A. Albrecht:** None. **A. Moszczynska:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

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

Support: NSF Grant DGE-1414475

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Psi Chi Grant

Title: Acute d-amphetamine unmasks the effects of adolescent methylmercury exposure on delay discounting in mice

Authors: ***S. BOOMHOWER**, M. NEWLAND;
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Abstract: The developing fetus is particularly vulnerable to the neurobehavioral effects of exposure to methylmercury (MeHg), an environmental neurotoxicant that bioaccumulates in fish, but the consequences of exposure during the adolescent period remain virtually unknown. The current experiments were designed to assess the effects of adolescent MeHg exposure on delay discounting (i.e., preference for small, immediate reinforcers over large, delayed ones) and sensitivity to amphetamine (a dopamine agonist) using a mouse model. Thirty-six male C57BL/6n mice were exposed to 0, 0.3, or 3.0 ppm mercury (as MeHg) via drinking water from postnatal day 21 to 59, the murine adolescent period. As adults, mice lever-pressed for a .01-cc droplet of milk delivered immediately and four .01-cc droplets delivered after a series of delays for 35 sessions. Then a dose-response determination of d-amphetamine (i.p.; 0.1 - 1.7 mg/kg) followed. Adolescent MeHg exposure impaired the acquisition of delay discounting relative to controls. d-Amphetamine increased preference for the larger-later reinforcer to a lesser extent in MeHg-exposed subjects compared to control mice. The current study is the first to demonstrate that adolescence is a period in which MeHg exposure may have long-lasting neurobehavioral consequences.

Disclosures: **S. Boomhower:** None. **M. Newland:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

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

Support: NIH Grant R03 DA029480

NIH Grant P50 DA026306

NIH Grant R01 MH087332

NIH Grant R25 MH081482

Title: Neuroaids on methamphetamine and cART: depletion of neuronal energy supplies

Authors: *A. B. SANCHEZ[1], G. P. VARANO[1], M. KINOMOTO[1], C. M. DE ROZIERES[1], R. MAUNG[1], I. C. CATALAN[1], C. C. DOWLING[1], N. E. SEJBUK[1], M. M. HOEFER[1], M. KAUL[1,2];
[1]Sanford-Burnham Med. Res. Inst., La Jolla, CA; [2]Dept. of Psychiatry, Univ. of California San Diego, San Diego, CA

Abstract: HIV-1 infection can cause neurological damage and lead to the development of HIV-associated neurocognitive disorders (HAND) despite advanced antiretroviral therapy (ART). In fact, accumulating evidence suggests that ART can itself exert neurotoxic effects. In addition, the use of recreational drugs, in particular methamphetamine (METH), in combination with HIV-1 infection seems to aggravate cognitive impairments and may compromise the efficacy of antiretroviral (ARV) drugs. The combined effect of recreational and therapeutic drugs and virus on the brain is poorly understood. In the present study, we exposed mixed neuronal-glial cerebrocortical cells to several ARVs and METH in the presence or absence of HIV-1 gp120 protein for 24 h and 7 days. First, we assessed neuronal injury and death in fixed cells using specific markers for neuronal dendrites (MAP-2) and pre-synaptic terminal (synaptophysin) in combination with nuclear DNA staining and fluorescence microscopy. When we analyzed the effect of cART compounds representing four different pharmacological categories (AZT, NVP, SQV and 118-D-24) in the presence or absence of gp120, none caused a significant neuronal injury in 24 h. However, after 7 days in the presence of gp120, only neuronal synapses were compromised, while MAP-2 positive dendrites were spared. We also analyzed potential cellular and neuronal toxicity using an ATPlite assay, a well established method used in large-scale drug screening for evaluation of cytotoxicity. The ATP assay similarly indicated that the occurrence

of cytotoxicity was context-specific for each of the drugs or combinations thereof. To recover from energetic stress, autophagy is generally activated in order to preserve the vital cellular functions. To address this hypothesis activation of AMPK, and processing of LC3 and p62 were assessed. Our results indicated that autophagy is induced during exposure of cerebrocortical cells to METH, ARVs and gp120 but fails to restore normal levels of cellular ATP. Altogether, our findings indicate that the overall positive effect of cART in HIV infection is accompanied by some neurotoxicity which is aggravated in the presence of abused drugs.

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Poster

692. Methamphetamine and Drug Induced Toxicity

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

Support: NIH Grant R00ES017781

Title: Brain iron loading impairs dopaminergic function and promotes ADHD-like behavior

Authors: ***Q. YE**[1], J. CHANG[1], M. HAN[1], Y. LI[1], H. ALSULIMANI[1], A. V. MENON[1], R. DETH[2], J. KIM[1];
[1]Northeastern Univ., Boston, MA; [2]Nova Southeastern Univ., Fort Lauderdale-Davie, FL

Abstract: Mounting evidence has indicated that elevated levels of heavy metals, such as lead and mercury, alter epigenetic regulation and dopaminergic function and predispose to the development of mental illnesses, including attention deficit hyperactivity disorder (ADHD), schizophrenia and autism. While iron overload is associated with the progression of neurodegenerative diseases, the role of brain iron loading in psychiatric disorders is largely unexplored. To investigate the influence of brain iron loading on emotional behavior and redox-methylation status, the H67D HFE-mutant mice, a mouse model of brain iron loading, and their control wild-type mice (mixed background; 8-10 wk old) were tested in an open field for overall activity and elevated plus maze for anxiety/impulsivity behavior. Following behavioral testing, brain metal levels were determined by inductively coupled plasma mass spectrometry. Brain levels of thiol species, including glutathione (GSH and GSSG) and S-adenosylmethionine (SAM) and -homocysteine (SAH), were quantified by HPLC. Expression levels of dopamine-

associated proteins were determined by western blot analysis. H67D mutant mice displayed elevated brain iron levels by 20% ($p=0.001$) with normal levels of copper and zinc. H67D mice exhibited hyperactivity (67% increase, $p=0.007$), stereotypy (80%, $p<0.001$) and impulsivity-like behavior (70%, $p<0.001$) compared with age-matched wild-type mice. This ADHD-like behavior was unique to brain iron loading because animals with systemic iron overload, but with normal brain iron, did not show abnormal behaviors. H67D mice also down-regulated tyrosine hydroxylase by 26% ($p=0.008$) and dopamine D1 receptors by 22% ($p=0.043$) and up-regulated dopamine D2 receptors by 262% ($p=0.021$) in the brain. Brain dopamine levels were reduced by 24% ($p=0.025$). Moreover, in the H67D brain the levels of isoprostane, a marker of oxidative stress, were increased by 53% ($p=0.017$) and activity of mitochondrial superoxide dismutase, an essential anti-oxidant enzyme, was down-regulated by 15% ($p=0.026$). In addition, GSH/GSSG ratios were decreased by 38% ($p<0.001$) and SAH levels were increased by 13% ($p=0.015$). These results demonstrate decreased anti-oxidant reserves and reduced methylation capacity in brain iron loading and further suggest that iron accumulation in the brain could promote ADHD-related neurochemical and behavioral changes. Our findings also provide a molecular basis for treatment of metal-related psychiatric and affective disorders.

Disclosures: **Q. Ye:** None. **J. Chang:** None. **M. Han:** None. **Y. Li:** None. **H. Alsulimani:** None. **A.V. Menon:** None. **R. Deth:** None. **J. Kim:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

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

Support: Intramural Funds from Centers for Disease Control and Prevention

Title: Phenotype comparisons of ALDH1L1 BAC-TRAP mice under control and neurotoxic (MPTP) conditions

Authors: ***K. A. KELLY**, A. R. LOCKER, L. T. MICHALOVICZ, D. B. MILLER, J. P. O'CALLAGHAN;
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Abstract: A central problem in neurotoxicology is detecting selective and unpredictable damage to specific cells produced by toxic agents/mixtures. With few biomarkers of damage known, evaluating astrogliosis by measuring GFAP overcomes this problem as reactive astrocytes show

the location of toxicant-induced damage occurring anywhere in the CNS. Thus, determining specific *in vivo* transcriptomic profiles of astrocytes under control and reactive conditions will identify additional astrogliosis biomarkers. Heintz and Greengard (2008) introduced BAC-TRAP (translating ribosome affinity purification) technology that allows the characterization of the actively translating transcriptome of a particular cell type. Using multiple models of neurotoxicity we confirmed that ALDH1L1 is an astrocyte specific "housekeeping" gene/protein. Thus, ALDH1L1 BAC-TRAP (ALB-T) mice can be used to characterize the transcriptome of astrocytes under various conditions. Here, we characterize the phenotype of the 0, 1, and 2 copy ALB-T mouse (18 - 26 weeks old) and C57BL6/J (WT) mice, in both sexes, under control conditions and in response to neurotoxic damage using the well characterized dopaminergic neurotoxicant MPTP (12.5 mg/kg, s.c.) at 72 hrs post dosing. Brain, thymus, spleen, liver, kidney, adrenal gland, testes, uterus and ovaries were removed and weights recorded. Additionally, the olfactory bulbs, cerebellum, hypothalamus, hippocampus, striatum, cortex, midbrain, brain stem and pituitary brain regions were dissected and weights recorded. Striatum was analyzed for astrogliosis (GFAP), nerve terminal damage (tyrosine hydroxylase (TH) and dopamine (DA) and results indicate no basal differences in GFAP, TH, or DA in control 0, 1, or 2 copy ALB-T when compared to WT mice. Although ALB-T mice showed greater increases in GFAP protein after MPTP exposure when compared to WT the TH and DA decreases were less than those of WT mice. Striatal tissue obtained at 48 hrs post MPTP was subjected to TRAP to isolate actively translating RNA in astrocytes and changes in the transcriptome determined by microarray (Illumina) and the dataset interrogated with Ingenuity Pathway Analysis. MPTP induced robust transcriptome changes revealing genes both expected to change with astrogliosis and not previously attributed to astrocytes. Our data indicate that BAC-TRAP technology can be used to identify additional biomarkers of astrogliosis and will aid in characterizing various astrocyte phenotypes.

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Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

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Program#/Poster#: 692.08/L31

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

Support: USFDA/NCTR E07394.01

Title: Histopathological indices of rotenone-evoked dopaminergic toxicity: neuroprotective effects of acetyl-L-carnitine

Authors: *Z. K. BINIENDA, B. GOUGH, S. SARKAR;
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Abstract: Exposure to the natural pesticide, rotenone, a potent mitochondrial toxin, leads to degeneration in striatal nerve terminals and nigral neurons. Rotenone-induced behavioral, neurochemical and neuropathological changes in rats mimic those observed in Parkinson's disease. Here, the protective effects of acetyl-L-carnitine (ALC) against dopaminergic toxicity after repeated exposures to rotenone were evaluated using immunolabeling methods. Adult male Sprague-Dawley rats were injected i.p. with rotenone alone (1 mg/kg) or rotenone with ALC either 10 or 100 mg/kg(ALC10, ALC100, respectively) once daily on days 1,3,5,8,10,12,15,17,19,22,24, 26,29,31,33, and 37. Control rats received either ALC at 100 mg/kg or vehicle (30% Solutol HS 15 in 0. 9% saline) injections. Animals were weighed on injection days and monitored daily. Rats were perfused after treatment with 4% paraformaldehyde and immunohistochemical analyses of tyrosine hydroxylase (TH) and the dopamine transporter (DAT) were performed in the caudate-putamen (CPu) and the substantia nigra pars compacta (SNc) in order to estimate loss of TH and transporter damage. Additionally, the effect of ALC on rotenone-induced microglial or astrocytic hypertrophy was also evaluated. It was found that ALC at 10 and 100 mg/kg prevented the loss of DAT and TH in the midbrain and also prevented the activation of both microglia and astroglia after rotenone treatment, thus, demonstrating the neuroprotective effects of ALC in rotenone-evoked dopaminergic neurotoxicity.

Disclosures: Z.K. Binienda: None. **B. Gough:** None. **S. Sarkar:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

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Program#/Poster#: 692.09/L32

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

Support: UNAM, PAPIIT IN 203912-3

Title: Rotenone affects the nigral dopaminergic system more readily when the exposure occurs at prenatal and early postnatal life than when it occurs at gestation, lactation or adulthood

Authors: *M. GÓMEZ-CHAVARÍN[1,2], J. RAMIREZ SANTOS[3], P. PADILLA[4], G. PERERA-MARIN[6], G. GUTIERREZ-OSPINA[5];
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Abstract: Parkinson´s disease (PD) may ensue following the exposure to herbicides, such as rotenone, throughout life. It is yet unclear whether rotenone affects equally/differently the developing and adult brain's dopaminergic system. Hence, in this work we compare the effects of rotenone upon the adult nigral dopaminergic neurons following gestational and/or early postnatal and adult exposure. Methods: The experiments were conducted in two and twelve months old male Wistar rats that were exposed to rotenone during gestation, lactation or both. Also, rotenone exposure occurred in twelve months old male rats. Serum samples were withdrawn to corroborate the presence of rotenone through high performance liquid chromatography. Dopaminergic neuron damaging was verified using tyrosine hydroxylase immunocytochemistry. The presence of Parkinsonian motor symptoms was evaluated through the use of the inclined beam before animal sacrifice. Results: In our experimental series rotenone exposure decreased the number of dopaminergic neurons regardless of the animal age when the exposure occurs. Nonetheless, the magnitude of the dopaminergic neuron loss was greater in rat groups exposed during gestation and lactation and directly correlated with rotenone serum concentrations. Motor impairment was always more evident in rats exposed to rotenone during gestation and lactation. Conclusion: Our results support that rotenone exposure during prenatal and early postnatal development is much more harmful for the nigral dopaminergic system than the exposure occurring at gestation, lactation or adulthood alone.

Disclosures: M. Gómez-Chavarín: None. **J. Ramirez Santos:** None. **P. Padilla:** None. **G. Perera-Marin:** None. **G. Gutierrez-Ospina:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

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Program#/Poster#: 692.10/L33

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

Support: Construction Fund for Key Subjects of Hunan Province (2013)

Title: Mitophagy alleviated CPF induced neurotoxicity in SH-SY5Y cells by PINK1/Parkin pathway

Authors: *J. ZHANG[1], H. DAI[2], L. ZHAO[2];
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Abstract: A growth body of evidence suggests that the pesticides are associated with the neurological disorders. Organophosphate chlorpyrifos (CPF) is the widely used pesticide throughout the world. Mitochondrial damage was reported to play a key role in CPF-induced neurotoxicity. Mitophagy, the selective autophagic elimination of mitochondria, is an important mitochondrial quality control mechanism. In order to explore the CPF-caused mitochondrial damage and the effect of mitophagy on CPF-induced neuroapoptosis, we examined the PTEN-induced putative kinase 1 (PINK1)/Parkin-mediated mitophagy and the casepase-3 related apoptosis by using dopaminergic SH-SY5Y cells. SH-SY5Y cells were treated with five different concentrations (12.5μM, 25μM, 50μM, 100μM, 200μM) of CPF for 6, 12, 24 or 48 h. Microscopic observations and cell counting kit-8 (CCK-8) assay were used to evaluate the morphological changes and cell viability respectively. Mitochondrial morphology was determined by immunofluorescence staining with an antibody against mitochondrial protein TIM23. The conversion of non-fluorescent chloromethyl-DCFDA to fluorescent DCF was used to monitor intracellular reactive oxygen species (ROS) production according to the instruction. Fluorescent microscopy was used to examine the colocalization between mitochondria and autophagosome. microtubule-associated protein1 light chain 3 (LC3)-GFP and mito-Dsred were used to label autophagosome and mitochondria, respectively. SH-SY5Y cells were transfected with Parkin-GFP plasmids and mito-DsRed plasmids to determine whether CPF treatment caused the accumulation of Parkin on mitochondria in SH-SY5Y cells. The effects of Parkin-knock down and overexpression on CPF-induced apoptosis were both examined. We found that CPF treatment caused mitochondrial fragmentation, excessive ROS generation and mitochondrial depolarization; these further inhibited cell viability and increased casepase-3 related apoptosis. CPF treatment also dramatically induced colocalizaton of mitochondria and LC3, decreased the level of mitochondrial protein, and promoted Parkin accumulation and PINK1 stabilization, these indicate PINK1/Parkin-mediated mitophagy. Furthermore, Parkin knock down dramatically increased CPF-induced neuroapoptosis. Instead, overexpression of Parkin markedly alleviated CPF-induced neuroapoptosis. Our study demonstrated for the first time, that PINK1/Parkin-mediated mitophagy may play a protective role against CPF-induced neuroapoptosis and enhancing mitophagy may be a useful therapeutic strategy for treating CPF-induced neurological disorders.

Disclosures: J. Zhang: None. **H. Dai:** None. **L. Zhao:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.11/L34

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

Support: Department of Veteran Affairs CDA Award BLR&D #BX001677

Title: Investigating *in vitro* neurotoxicity of antiretroviral agents

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Abstract: Introduction: HIV neurocognitive disorders (HAND) affect approximately 50% of HIV patients that receive antiretroviral agents (ARVs). Studies of HIV patients taking ARVs showed measurable neurocognitive improvements when ARVs were temporarily discontinued. Such studies suggest ARVs may contribute to HAND. Additionally, studies of the effects of ARVs on mixed neuron/glia cultures showed most agents tested decrease neuron density at concentrations lower than therapeutic plasma concentrations as judged by the neuron-specific microtubule associated protein 2 (MAP2). Methods: Here we refine and extend the *in vitro* analysis of ARV effects on neurons by using mature (DIV 16), glia-reduced, primary rat cortical neuron cultures. We assessed the effects of increasing concentrations of commonly prescribed ARVs (amprenavir, AZT, dideoxyinosine, efavirenz, indinavir, lamivudine, stavudine, tenofovir) on neuron viability in high density (188K cells/cm^2) and morphology (dendrite length and extent of branching) in low density (6.4K cells/cm^2). Low density cells were suspended above a glia feeder layer. Viability (via Cell-Titer Blue, Promega, USA) and morphology (via MAP2 immunofluorescence) were assessed 24 hours after agent addition. Results: Cell viability assays showed efavirenz caused significant toxicity. Viability of 50% was reached at about 50 μM, and 0% viability occurred at 100 μM. Immunofluorescence imaging showed significantly lower total dendritic length compared to vehicle-treated cells for efavirenz (≥20 μM), dideoxyinosine (≥200 μM), and tenofovir (≥10 μM) ($p<0.05$, post-hoc Dunn's test). Conclusions: Total dendritic length was a more sensitive measure than cell viability for revealing differences between vehicle- and ARV-treated neurons. Whereas most ARVs tested did not alter neuron viability or morphology, some agents (efavirenz and tenofovir) significantly reduced dendritic length at concentrations within an order of magnitude of the therapeutic plasma concentration in HIV patients taking these agents. Additional studies of neurotoxic mechanisms of ARVs may be useful to optimally treat HIV patients with cognitive disorders.

Disclosures: V.T. Ciavatta: None. **C. Cron:** None. **S. Teng:** None. **W.R. Tyor:** None. **P. Garcia:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.12/L35

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

Title: Neurodegenerative potential of the aqueous leaf extract of ocimum gratissimum: a histological and biochemical study

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Abstract: Ocimum gratissimum is an herbaceous perennial shrub which is widely distributed in many regions. It is consumed in food as seasoning locally in Nigeria. In the present study, the effect of the acute administration of the aqueous leaf extract of Ocimum gratissimum (AeOG) on prefrontal cortical neurons was checked to assess its neurotoxicity potential. Thirty adult male Wistar rats weighing between 190-210 g were divided into 5 groups (n=6). Group A (control) received 1 ml of normal saline (p.o), groups B-E received 100, 200, 300 and 400 mg/kg AeOG (p.o) respectively. Treatment lasted for fourteen days. Twenty-four hours after treatment, animals were sacrificed and their brains were removed. The prefrontal cortices neuronal morphology was studied using haematoxylin and eosin (H&E) stain; while activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) were assayed in the cerebral homogenate. AeOG administration at doses 300 and 400 mg/kg cause neuronal fragmentation with significant ($P<0.05$) increases in the activities of cerebral ACP and ALP. Our findings show that the acute use of AeOG caused neuronal fragmentation which may leads to onset of neurodegenerative diseases and affect cognitive and executive functions of the prefrontal cortex.

Disclosures: M.I. Ajibola: None. **R.B. Ibrahim:** None. **A.A. Safiriyu:** None. **A.T. Etibor:** None. **M.A. Mustapha:** None. **A. Imam:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.13/L36

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

Support: CB183867

CB180851

Title: The impairs on mitochondrial membrane potential, cellular dysfunction and circling behavioral in the mechanism of xanthurenic acid toxicity *in vivo*

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Abstract: Xanthurenic acid (XA), a metabolite of tryptophan degradation is synthesized through 3-hydroxykynurenine transamination by kynurenines aminotransferases on both cytoplasm and mitochondria. The formation of XA is thought to be the main route to prevent the accumulation of the potential toxin 3-HK. However, some studies have shown that XA induces cell death and produces free radicals as a result of its interaction with metals. On the contrary, some reports show that XA acts as a scavenger and it is able to prevent the lipid peroxidation induced by iron. Dual action of this metabolite is important if we consider that can cross the blood brain barrier and that alteration of XA levels are related with some pathologies. The aim of this study was to investigate the effect of a single intrastriatal injection of XA on mitochondrial function, mitochondrial membrane potential, ROS production, circling behavior and cell death. First, we evaluated the effect of intrastriatal administration of XA (5-50 nmol) in rats, on cellular function, ROS production and mitochondrial membrane potential. Our results showed that after 5 days of XA administration, the mitochondrial membrane potential and MTT reduction were decreased while ROS production was not increased. Interestingly, circling behavior and cell death were increased on a concentration dependent- manner after five days of XA administration. Together, these data suggest that this kynurenine exerts toxic effects through different mechanisms including impairing cellular energetic metabolism, which can leads to cellular degeneration; this mechanism seems to be independent of its pro-oxidant ability.

Disclosures: J.G. Reyes Ocampo: None. **B. Pineda:** None. **D. González-Esquivel:** None. **L. Sánchez-Chapul:** None. **C. Ríos:** None. **A. Jiménez-Anguiano:** None. **D. Silva-Adaya:** None. **V. Perez-de la Cruz:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.14/L37

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

Support: CNPq

FAPERGS

Title: Acute and subchronic neuropharmacological, genotoxic, and mutagenic profiles of a Brazilian fruit compound, garcinielliptone FC, in mice

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Abstract: The Amazon region is known to possess a great diversity of natural species, including fruit rich in vitamins and minerals. However, little is known about the biological properties of these species. Garcinielliptone FC (GFC) is a compound isolated from a fruit widely consumed as food in the North and Northeast of Brazil. This study evaluated pharmacological and toxicological effects of GFC, a polyisoprenylated benzophenone isolated from Platonia insignis Mart. fruits, by using behavioral models and genotoxic/mutagenic assays. The effects of GFC were evaluated in mice after acute and subchronic intraperitoneal (i.p.) treatments. The behavioral tests conducted were the motor activity, open field, tail suspension, and rotarod tests. The genotoxic effects were assessed using the comet assay and micronucleus test. The acute toxicity evaluation suggested an LD50 between 50 and 300 mg/kg. No impairment was observed in the rotarod test after subchronic treatment with GFC at all tested doses (2, 10, and 20 mg/kg). GFC did not affect locomotion in mice after acute treatment, and the latency to start locomotion, rearings, or crossings did not change compared to that in the control group, even after 28-day administration of the same doses. GFC did not affect the immobility time in the tail suspension task after both treatments protocols used, and did not show genotoxic effects on the blood and cerebral cortex. However, there was a significant increase in index and frequency of damage in the liver at 20 mg/kg. GFC did not increase the micronucleus frequency in bone marrow assessed using the micronucleus test. These results suggest that GFC had no effect on motivation, exploration, or locomotion at the doses tested, as well as antidepressant-like activity. DNA damage to liver tissue was observed at only the highest dose of GFC (20 mg/kg), suggesting hepatotoxicity at this dose but no mutagenic potential was observed.

Disclosures: **J.N. Picada:** None. **V.R. Coelho:** None. **L.S. Prado:** None. **C.G. Vieira:** None. **L.P. Souza:** None. **G.C. Gonçalves:** None. **P. Pfluger:** None. **M.T.C. Valle:** None. **M.B. Leal:** None. **E. Dallegrave:** None. **A.B.F. Ferraz:** None. **P. Pereira:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.15/L38

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

Support: NCTR E-7285

Title: Potential adverse effects of sevoflurane on developing monkey brain: from abnormal lipid metabolism to neuronal damage

Authors: ***C. WANG**[1], F. LIU[1], S. W. RAINOSEK[2], J. L. FRISCH-DAIELLO[3], T. A. PATTERSON[1], M. G. PAULE[1], W. SLIKKER, Jr.[1], X. HAN[3];
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Abstract: Sevoflurane is a volatile anesthetic that has been widely used in general anesthesia, yet its safety in pediatric use is of public concern. The present study sought to evaluate whether prolonged exposure of infant monkeys to a clinically-relevant concentration of sevoflurane is associated with any adverse effects on the developing brain. Infant monkeys were exposed to 2.5% sevoflurane for 9 hours, and frontal cortical tissues were harvested for DNA microarray, lipidomics, Luminex protein and histological assays. DNA microarray analysis showed that sevoflurane exposure resulted in 576 differentially expressed genes (DEGs) in the monkey brain. In general, these genes were associated with nervous system development, function, and neural cell viability. Notably, a number of DEGs were closely related to lipid metabolism. Lipidomic analysis demonstrated that critical lipid components, [e.g., phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylglycerol (PG)] were significantly down-regulated. Luminex protein analysis indicated abnormal levels of cytokines in sevoflurane-exposed brains. Consistently, Fluoro-Jade C staining revealed more degenerating neurons after sevoflurane exposure. These data demonstrate that a clinically-relevant exposure to sevoflurane is capable of inducing and maintaining an effective surgical plan of anesthesia in the developing nonhuman primate and that a prolonged exposure of 9 hours resulted in profound changes in gene expression, cytokine levels, lipid metabolism, and subsequently, neuronal damage. Generally,

sevoflurane-induced neuronal damage was also associated with changes in lipid content, composition, or both; and specific lipid changes could provide insights into the molecular mechanism(s) underlying anesthetic-induced neurotoxicity and be sensitive biomarkers for the early detection of anesthetic-induced neuronal damage.

Disclosures: C. Wang: None. **F. Liu:** None. **S.W. Rainosek:** None. **J.L. Frisch-Daiello:** None. **T.A. Patterson:** None. **M.G. Paule:** None. **W. Slikker:** None. **X. Han:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.16/L39

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

Support: NCTR Experiment #7285

FDA/CDER

Title: The utility of a nonhuman primate model for assessing general anesthetic-induced developmental neurotoxicity: sevoflurane as test agent

Authors: *M. G. PAULE[1], S. LIU[1], X. ZHANG[1], R. CALLICOTT[1], G. NEWPORT[1], T. A. PATTERSON[1], S. M. APANA[2], M. S. BERRIDGE[2], M. P. MAISHA[3], J. P. HANIG[5], W. SLIKKER, Jr.[4], C. WANG[1];
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Abstract: To maximize the relevance of data generated in preclinical studies of general anesthetic-induced developmental neurotoxicity, the physiological status of the animal models employed must be maintained within normal ranges during experimental procedures. Depth of anesthesia should also be monitored when potential neuroprotective agents are used to insure that they do not diminish anesthetic efficacy. In addition, the identification of blood borne markers associated with anesthetic-induced neurotoxicity could prove valuable in providing easily obtainable metrics of adverse outcome(s). Physiological parameters were closely monitored during an 8-hour bout of sevoflurane- (2.5%) induced general anesthesia in postnatal day (PND) 5 or 6 rhesus monkeys. The depth of anesthesia was monitored using quantitative

electroencephalography (QEEG), and plasma samples collected during the exposure were analyzed for levels of several pro-inflammatory mediators. In addition, micro positron emission tomography (microPET) using the radiolabeled ligand [^{18}F]FEPPA, which binds to activated glial cells in the central nervous system (CNS), was performed one day following the exposure. Important physiological metrics (e.g., 02 saturation, blood glucose and pH, pCO2) observed in anesthetized monkeys (n = 8) were not significantly altered in comparison with those of control animals (n = 6). The co-administration of acetyl-L-carnitine (n = 7), which is postulated to be neuroprotective by stabilizing mitochondria, did not result in any significant change in the depth of anesthesia as indicated by QEEG. Increased production of two pro-inflammatory mediators (IL-6 and CCL-2) was detected in plasma near the end of the 8-hour sevoflurane exposure and enhanced uptake of [^{18}F]FEPPA was observed in the frontal cortical region the day following anesthesia, indicating reactive gliosis associated with presumed neurotoxic injury. The coincident presence of pro-inflammatory mediators in blood suggests that these markers may be used as indicators of anesthetic-induced developmental neurotoxicity. The data obtained here serve to demonstrate the usefulness of the nonhuman primate model in the evaluation of anesthetic-induced developmental neurotoxicity.

Disclosures: **M.G. Paule:** None. **S. Liu:** None. **X. Zhang:** None. **R. Callicott:** None. **G. Newport:** None. **T.A. Patterson:** None. **S.M. Apana:** None. **M.S. Berridge:** None. **M.P. Maisha:** None. **J.P. Hanig:** None. **W. Slikker:** None. **C. Wang:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

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

Support: Capes foundation, Ministry of Education of Brazil, Proc. #0407/13-5

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Title: Effects of manganese exposure on the dopaminergic system and antioxidant enzymes in Caenorhabditis elegans with mutations in AKT signaling pathway

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Abstract: Environmental contamination by metals is a risk factor for public health and the central nervous system (CNS) is one of the targets of these toxic agents. Manganese (Mn) is an essential metal, but in excess it may cause a syndrome analogous to Parkinson's disease, called manganism. Mn accumulates preferentially in basal ganglia and may induce oxidative stress and interfere with signaling pathways. In the nematode worm *Caenorhabditis elegans,* the kinases AKT-1, AKT-2 and Serum- and glucocorticoid-inducible kinase (SGK-1) participate in the insulin/insulin-like growth factor signaling pathway (IIS). This signaling pathway regulates worm longevity, metabolism and the action of transcription factors SKN-1 (the worm homologue of Nrf-2) and DAF-16 (homologue of FOXO). To investigate signaling pathways involved in Mn toxicity, wild type (N2) and loss-of-function mutant (for proteins of the AKT signaling pathway) *C. elegans* were used. The worms were exposed to Mn for 1 h at the L1 larval stage using concentrations of 2.5 to 100 mM Mn. Strains with loss of function in *akt-1*, *akt-2* or *sgk-1* had higher resistance to Mn compared to N2 in a survival test. This resistance may be related to the antioxidant response. The N2 worms had decreased levels of glutathione (GSH) after exposure to Mn, which did not occur in AKT mutants. qRT-PCR studies showed that Mn exposure induced an increase in the expression of the gene encoding the SKN-1 transcription factor in *akt-2* mutants, and γ-glutamine cysteine synthetase (GCS-1) antioxidant enzyme in *akt-1* mutants. Notably, the expression of *sod-3* (homologue of superoxide dismutase, SOD) was increased in the *akt-1* mutant worms independent of Mn treatment. Dopaminergic function was evaluated with the basal slowing response behavioral test. Dopaminergic neurons integrity was evaluated using worms expressing green fluorescent protein (GFP) under the dopamine transporter (DAT1) promoter. However dopaminergic neurons showed analogous degeneration in N2 and mutant worms. These results suggest that AKT signaling pathway participates in Mn-induced toxicity in *C. elegans* due to its role in antagonizing the transcription factors SKN-1 and DAF-16, which are important for the production of antioxidant enzymes in the worms. However, this effect is not present in the dopaminergic neurons. This study demonstrates the importance of intracellular signaling pathways to the antioxidant response induced by Mn and establishes AKT as an important research focus for further understanding of the mechanisms of Mn-induced toxicity.

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Poster

692. Methamphetamine and Drug Induced Toxicity

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Program#/Poster#: 692.18/L41

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

Support: CNPq, 406807/2013-2

CNPq, 300966/2014-8

Title: Oxidative stress as a key event in malaoxon-induced neurotoxicity in primary cell cultures of cortical neurons

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Abstract: Aim: Organophosphates (OPs) comprise many of the most common agricultural and commercial pesticides used worldwide. The acute toxicity of OPs is primarily caused by the inhibition of acetylcholinesterase (AChE). However, there is now substantial evidence that exposure to levels that produce no explicit signs of acute toxicity has been associated with neurobehavioral and cognitive deficits. Nevertheless, molecular mechanisms related to this non-cholinergic neurotoxicity are not yet fully understood. This study aimed to investigate the molecular mechanisms of non-cholinergic neurotoxicity induced by exposure to malaoxon (Mx), the active metabolite of the pesticide Malathion, in an *in vitro* model of primary cell cultures of cortical neurons. Methods: Primary culture of cortical neurons were incubated with different concentrations of Mx (0.01 - 100 μM) and cell viability was determined at 24 and 48h or 6 days *in vitro* (DIV) by the MTT method. AChE enzyme activity was determined by the production of thiocholine from the hydrolysis of acetylthiocholine. General reactive oxygen species (ROS) were measured using the fluorescent probe 2', 7' diclorodiidrofluoresceína acetate (DCFH-DA). Superoxide anion production was determined by using fluorescent dye dihydroethidium (DHE) and mitochondrial membrane potential (MMP) by using the cell permeable fluorescent dyes rhodamine 123. Results: A significant decrease in cell viability was observed at 48 h after Mx treatment (concentrations of 1, 10 and 100 μM). However, a significant increase in pro-oxidative stress-related parameters was already observed in earlier times (1h). Pretreatment of the cells with the antioxidant ascorbic acid (200 μM) prevented the oxidative damage, likewise exhibited partial neuroprotective effects by preventing cell death induced by Mx. Mx treatment significantly decreased AChE activity after 24h of treatment. The incubation with Pralidoxime (600 μM), the oxime most used in the clinical, reversed this inhibition (at the Mx concentrations of 0.01 to 1 μM), but did not reverse the cell death induced by Mx. Conclusion: These results indicate the occurrence of oxidative stress in neuronal cortical cells soon after Mx exposure,

which seems to be critical for Mx-induced neurotoxicity. The present results also disconnect Mx-induced neurotoxicity from its classical anticholinesterasic effects.

Disclosures: **D.K. Venske:** None. **A.A. dos Santos:** None. **C. Suñol:** None. **M. Farina:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.19/L42

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

Title: Potential neuroprotective effect of docosahexaenoic acid in rotenone-treated rats

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Abstract: Background: The causes of neuronal death in neurodegenerative diseases are currently unknown. However, it has been suggested that mitochondrial dysfunction can promote neuronal death and the symptoms of neurodegenerative diseases. Among the experimental models used, rotenone is one of the most widely used to inhibit respiratory chain's complex l. leading dopaminergic neuronal death resulting in a Parkinson disease-like. Furthermore, it has been reported that the essential polyunsaturated fatty acids (EPUFA) have important effects on the nervous system. In this regard, it has been shown that docosahexaenoic acid (DHA) has a neuroprotective effect on dopaminergic neurons. Also, in recent years, it has been shown that the EPUFA has an effect on mitochondrial biogenesis. Aim: The aim of this study was to evaluate the potential neuroprotective effect of DHA on mitochondrial dysfunction induced by rotenone in rat striatum and midbrain. Methods: Eighty male Wistar rats were assigned under the following conditions: pretreatment of DHA (35 mg/kg/day) for 7 days + rotenone for 8 and 14 days to analyze the exploratory activity, tyrosine hydroxylase (TH) enzyme and, in isolated mitochondria, complex l activity, respiratory control index, transmembrane potential and ATP synthesis capacity. Results: DHA did not attenuate the damage in the exploratory activity at 14 days of treatment with rotenone. In addition, at 8 days, DHA prevented damage of rotenone in the exploratory activity (40%), and attenuated both the histological damage and the decreased in the TH protein content induced by rotenone in striatum and midbrain. On the other hand, DHA was unable to attenuate the rotenone-induced mitochondrial dysfunction in striatum and midbrain Conclusion: DHA administration has a neuroprotective effect after 8 days of rotenone

treatment but this effect was not related to the attenuation of rotenone-induced mitochondrial alterations.

Disclosures: **N. Serrano:** None. **V. Pérez de la Cruz:** None. **A. Jiménez-Anguiano:** None. **J. Pedraza-Chaverri:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.20/L43

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

Title: Pathophysiology and experimental therapeutics of tetramethylenedisulfotetramine (tetramine) poisoning

Authors: *D. L. SPRIGGS, S. DEBUS, R. KREMPEL, J. SKOVIRA;
USAMRICD, Gunpowder, MD

Abstract: Tetramethylenedisulfotetramine (TETS) is a highly toxic cage-convulsant that has been banned from use as a pesticide worldwide. Despite this ban, accidental poisonings as well as intentional mass poisonings have been reported. The neurotoxic effects of TETS result from irreversible binding to GABAA receptors which blocks chloride ion influx and facilitate neuronal activation leading to seizures and convulsions. The mechanisms of TETS toxicity remain poorly understood and there is currently no adequate medical countermeasure to mitigate its effects. This study was designed to characterize cardiorespiratory and cortical changes throughout the course of TETS poisoning and evaluate potential therapeutic countermeasures. One week prior to experimentation guinea pigs were instrumented to monitor lead II electrocardiogram, electroencephalogram, and body temperature. A plethysmography chamber was used to measure respiratory rate, tidal volume and minute volume. Survival was assessed 24 hours post exposure. Following a 15 minute control recording, each animal received a single intraperitoneal 2LD50 dose of TETS. The first sign of poisoning was a drop in heart rate (from 281.9 ± 9.1 to 211.8 ± 10 beats per minute) within minutes of intoxication. All animals exhibited an initial brief period of cortical seizure activity and convulsions starting 5.2 ± 1.4 min post exposure which subsided spontaneously (1.2 ± 0.2 min duration). In control animals several periods of seizure activity or progression to status epilepticus occurred. The average time to death in control animals was 67.8 ± 23.6 min post TETS. In the treatment groups therapies were administered following the end of the initial seizure. Phenobarbital (PHB) therapy was compared to the commonly used benzodiazepine, Diazepam. A 37.5% survival rate at 24 hours was

observed following a single dose of PHB (100 mg/kg, ip). Diazepam (5 mg/kg, ip) produced marginally better results, with 50% survival at 24 hours. Neither therapy was completely effective in blocking seizure activity. In addition, both groups had periods of bradycardia, bradypnea and motor dysfunction, including dystonia, ataxia and convulsions. The addition of a muscarinic antagonist to PHB treatment increased 24 hour survival. Administration of the peripherally acting Atropine methyl nitrate (2 mg/kg, im) with PHB improved the 24 hour survival rate to 50%. Co-administration of centrally acting Atropine Sulfate (2 mg/kg, im) with PHB increased the 24 hour survival rate to 75% suggesting central antagonism of muscarinic receptor signaling is important to increasing survival following TETS poisoning.

Disclosures: **D.L. Spriggs:** None. **S. DeBus:** None. **R. Krempel:** None. **J. Skovira:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.21/L44

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

Support: CONACYT 152842

PAPIIT 202013

Fellowship from CONACYT 619857

Title: Dose and gender effects of chronic arsenic exposure in locomotor activity and lipid peroxidation in the dopaminergic system of C57BL/6 mice

Authors: R. CONTRERAS-LÓPEZ[1], M. DÍAZ-MUÑOZ[2], M. GIORDANO[1], *V. RODRIGUEZ CORDOVA[3];
[1]Dept. de Neurobiología Conductual y Congnitiva, [2]Dept. de Neurobiología Celular y Mol., [3]Inst. de Neurobiologia UNAM, Juriquilla, Mexico

Abstract: Arsenic is a highly toxic metalloid, chronic exposure through drinking water (DW) is associated with cancer of internal organs and nervous system dysfunctions. In animal models, As exposure causes alterations of dopaminergic markers and antioxidant response in the brain. Here, we assessed the effects of As exposure on spontaneous locomotor activity and lipid peroxidation in the nigrostriatal and mesolimbic dopaminergic systems in female and male mice. In order to answer our question, two-month old C57BL/6 male and female mice were daily exposed to 0.05, 0.5 or 50 mg As/L DW during for 6 months. Spontaneous locomotor activity was evaluated

monthly, and at the end of the treatment period thiobarbituric acid reactive species, and conjugated dienes were determined in striatum, nucleus accumbens and midbrain. We found that after four and six months of As exposure horizontal activity, and stereotypy number increased only in female mice exposed to 50 mg As/L. On the other hand, levels of conjugated dienes were higher in the nucleus accumbens and midbrain of male mice, and in nucleus accumbens of female mice exposed to 0.5 and 50 mg As/L in comparison to control animals; no alterations in TBARS levels were found. In conclusion, chronic arsenic exposure disrupts spontaneous locomotor activity and alters lipid peroxidation in a gender dependent manner in dopaminergic areas of the brain. We thank Soledad Mendoza, Hector and Dr. Olivia Vázquez Martínez for their technical assistance.

Disclosures: R. Contreras-López: None. **M. Díaz-Muñoz:** None. **M. Giordano:** None. **V. Rodriguez Cordova:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.22/M1

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

Support: GDRI Neuro CNRS/CNRST

Title: Negative effects of a mixture of lead and aluminum on neurodevelopmental behavior in rats

Authors: *F. CHIGR[1], K. EL MANJA[2], H. MALQUI[2], N. OUASMI[2], M. NAJIMI[2];
[1]Biol., Sultan Moulay Slimane University, Fac. of Scien, Beni Mellal, Morocco; [2]Fac. of Sci. and Techniques, Beni Mellal, Morocco

Abstract: The developing central nervous system is particularly vulnerable to environmental toxicants such as heavy metals (HM). While acute and chronic poisoning of each metal has been extensively studied, the effects of joint exposures to these metals are poorly understood, even though this exposure scenario more accurately reflects the real world situation. HM exposure is not only a health concern for adults but has also been shown to exert deleterious effects on the health of the fetus, newborn and child. In this study, we have examined the effect of a mixture of low doses of lead (Pb): 0.05% and aluminum (Al): 0.12% diluted in drinking water on the growth and development in the rat. At postnatal day (PN) 0, all offspring were counted; weighed, and examined for external malformations. The following physical developments were noted: hair

appearance, incisor eruption, and bilateral eye opening. These parameters are useful indicators of rat fetal development. First of all, prenatal and postnatal exposure to Pb-Al significantly increased the body weights of pups compared to normal pups. Concerning behavioural tests, when Cliff avoidance is applied, latency to win the test was significantly higher in treated rats compared to control rats during all recordings, on postnatal days (PN) 7 and 9. Furthermore, latency to jumping in cage appeared higher in treated rats compared to control rats, but was not statistically significant. Similarly, choice cage test is not different between treated and controls. In conclusion, administration of HM to dams during the entire gestation period affects behavior and development in pups. The observed effects were a delay in opening eyes and incisor eruption as well as behavioral developments and an alteration in the rate of success behavior. The results suggest that HM even given in low doses have a significant effect on the development of behavioral patterns, orientation and motor coordination and function. They also suggest significant growth retardation. These results indicate clearly that the developing brain is especially vulnerable to HM and that vulnerability could have consequences that extend into adulthood.

Disclosures: **F. Chigr:** None. **K. El Manja:** None. **H. Malqui:** None. **N. Ouasmi:** None. **M. Najimi:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

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Program#/Poster#: 692.23/M2

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

Support: NIMHD 5S21MD000101

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National Institutes of Health- NINDS U54NS083932

C06RR17571 – National Center for Research Resources

Title: The effects of methyl parathion on the nigrostriatal system

Authors: J. BEAN[1], *K. R. SHEPHERD[2];
[1]Biol., Morehouse Col., Atlanta, GA; [2]Pharmacology/Toxicology, Morehouse Sch. of Med., Atlanta, GA

Abstract: Organophosphate pesticides (OPs) such as methyl parathion (MeP) are the most widely used insecticides in the world. Due to their widespread use, a large number of poisonings occur every year in occupationally-exposed human populations and in the general population through the consumption of contaminated food and drinking water. The oxon is the mediator of acute OPs toxicity due to its ability to inhibit acetylcholinesterase activity in the nervous system and neuromuscular junctions. Recent studies have revealed several other targets of Ops, such as MeP, that disturb noncholinergic biological systems. The current study was undertaken to determine the effects of MeP on the nigrostriatal system. In the first study, we prepared primary neuron-glia co-cultures from striatum (ST), and the substantia nigra (SN) of C57BL6 mouse pups to determine MeP induced neurotoxicity, MeP significantly induced reactive oxygen species (ROS) in SN and ST cultures at concentrations ranging from 0.1 μM-10 μM. The findings suggest that the MeP is a potent inducer of ROS. In the MTT assay (a determination of mitochondrial activity in living cells); there was no significant change after 72 hours under serum supplemented conditions. However, there was a significant decrease in MTT absorbance after 72 hours under serum-free conditions. Glutathione peroxidase (GPx) activity was significantly decreased only in SN cultures. As a result, the SN may have reduced capacity to remove ROS as demonstrated by the reduction in GPx activity and increased ROS in these cultures after MeP exposure. In the second study, 6 month old C57BL6 mice were treated with i.p. injections of MeP. There was a decrease in tyrosine hydroxylase immunoreactive neurons in the SN. In addition, there was a significant decrease in striatal dopamine by30.1%, dihydroxyphenyl acetic acid (DOPAC) by 28%, and homvanillic acid (HVA) by 44.5%. Collectively, the findings from these studies demonstrate that the SN and ST regions are vulnerable to the effects of MeP. This may have implications for MeP to alter nigrostriatal dopaminergic functioning similar to that observed in neurodegenerative diseases such as Parkinson's.

Disclosures: J. Bean: None. **K.R. Shepherd:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.24/M3

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

Title: Ultrastuctural changes in fetal mouse retina induced by two different doses of diazepam

Authors: *M. MARQUEZ-OROZCO, G. DE LA FUENTE-JUAREZ, A. MARQUEZ-OROZCO;
Univ. of Mexico (UNAM), Mexico, Mexico

Abstract: Our purpose was investigated if fetal retinal ultrastructure alterations of mice, are similar in a group (DZ1) which mothers (CD-1 strain mice) were injected with diazepam (DZ) (days 6 to 17) with daily doses of DZ (2.7 mg/kg/bw/sc), other (DZ 2) treated during same period with doses of DZ (1.0 mg/kg/bw/sc) that the DZ1 group. The control group received saline solution (S). A fourth group was not treated (NT). All females were euthanized with CO2 atmosphere the 16th day of gestation and the fetuses removed and perfused intracardiac with 1% paraformaldehyde their eye globes, were fixed with 2.5% glutaraldehido, were post fixed in OsO4 and embedded in epóxica resin. The semi fine sections were stained with toluidine blue and observed under comparative light microscope. The fine sections were contrasted with uranyl acetate and lead citrate and observed under a transmission microscope. In both DZ1 and DZ2 groups the neuroblastic layers and the photoreceptors show delay cellular differentiation. A greater nuclear density of the retinal cells was observed ($p<0.05$). The cells showing nuclei with heterochromatin atypically distributed. The outer nuclear layer presents rounded undifferentiated nuclei and numerous mitotic cells. The photoreceptors layer was very thin. The mitochondrion, the polyribosomes, the Golgi complex and the rough endoplasmic reticulum were more abundant in the photoreceptors from the DZ1 and DZ2 fetuses than in S an NT fetuses. The pigmentary epithelium of DZ1 and DZ2 was thickening, show disorganized microvilli and mitochondrion, abnormal accumulation of glycogen grains, vacuoles and abnormal phagosomes. S and NT groups exhibited neither ultrastructural difference in the outer nuclear layer of the retina and photoreceptors structure the appearance was normal. Such alterations could be attributed DZ inhibiting mitosis, actin and myosin synthesis and the modification of the metabolic pathways by central and peripheral type benzodiazepine receptors. Results show that the DZ administration from doses of 2.7 mg/kg/bw/sc or 1.0 mg/kg/bw/sc during gestation produces the seam type of ultrastructural changes in the fetal retina.

Disclosures: M. Marquez-Orozco: None. **G. De la Fuente-Juarez:** None. **A. Marquez-Orozco:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.25/M4

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

Title: Permanent histological and ultrastructural alterations of the mesencephalic structures in mice prenatally exposed to diazepam

Authors: *A. MARQUEZ-OROZCO, G. DE LA FUENTE-JUAREZ, M. C. MARQUEZ-OROZCO;
Univ. of Mexico (UNAM), Mexico 04510 DF, Mexico

Abstract: The present work was aimed at determining if prenatal exposition to diazepam (DZ) produces histological and ultrastructural alterations in the mesencephalic structure of adult mice. Two gestating CD-1 strain mice groups were injected daily sc from day 6 to 17. The first group (DZ) was treated with single daily DZ doses (2.7 mg/kg/bw) and the second group with saline solution (S). A third group was non-treated (NT). The offspring's were wet nursed by non-treated mice weaned and kept for 240 days. Animals of DZ, S and NT groups were deeply anesthetized, perfused with 10% formalin, the brain were removed, fixed, and embedded in paraffin. The histological sections were stained with Kluver-Barrrera stain for myelin and Bodian techniques. The histological sections were observed under comparative light photonic microscope. Other animals of DZ, S and NT groups were deeply anesthetized and perfused intra cardiac with 1% paraformaldehyde and mesencephalon fixed in 2.5% glutaraldehido, post-fixed in OsO4, and embedded in epoxy resin. The fine sections were contrasted with uranyl acetate and lead citrate and observed under a transmission microscope. In the red nucleus, the superior and inferior colliculi of DZ group was demonstrated the presence of small-sized neurons poor densely packed. The nuclei of the neurons were retracted and showed clumps of heterochromatin. The nucleoli were voluminous. The rough endoplasmic reticulum was frequently dilated and disorganized. The polyribosomes and Golgi complex were abundant. The neuropile were poorly differentiated, and were observed coarse, disoriented and few fibers with irregular myelin sheath. Non histological or ultrastructural differences were found between the S and NT groups. The alterations could be attributed to DZ induces modifications of metabolic pathways mediated by central and peripheral type benzodiazepine receptors. The permanent alterations also may be an epigenetic effect of the DZ because alter the RNA expression. The results show permanent histological and ultrastructural effects of prenatal exposure of DZ in the mesencephalon structures of mouse.

Disclosures: A. Marquez-Orozco: None. **G. De la Fuente-Juarez:** None. **M.C. Marquez-Orozco:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.26/M5

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

Support: CAPES/FMJ

PROAPARC/UNASP

Title: Morphoquantitative evaluation of fibroelastic components in basilar and middle cerebral arteries of rats exposed to passive smoking

Authors: *R. N. ISAYAMA[1], E. ROBELLO[2], N. L. FROIO[2], C. A. FABREGA-CARVALHO[2]; [1]UNICASTELO, Fernandopolis, Brazil; [2]Morphology and Basic Pathology, Faculdade de Medicina de Jundiaí (FMJ), Jundiai, Brazil

Abstract: Basilar and middle cerebral arteries represent a significant bloodstream to the brain. Preview studies have shown that smoking habit can affect vascular wall components. Hence, smokers have more risk for cerebrovascular diseases, such as stroke, with stenosis or aneurysm of cerebral blood vessels. Even minor alterations in cerebral arteries may affect brain metabolism and function. Inflammatory response of the vascular wall is a vulnerable point of smoking, with increased cytokines, leucocytes and metalloproteinases that affect collagen synthesis in the adventitious layers of cerebral arteries. Nevertheless, these correlations have not been completely understood in models of passive smoke yet. This study analyzed fibroelastic components of the basilar and the middle cerebral arteries through stereology in a model of passive smoking in rats. The study was approved by the institutional committee of ethics. Male wistar rats with 6 weeks old each, were assigned to form a control group (CG; n=10) and a smoke group (SG; n=10). The latter was exposed to smoke of 20 cigarettes, distributed in 90 days of exposure, equivalent to 10mg tar, 0.8mg nicotine and 10mg carbon monoxide. Animal handling was standardized for both groups. Acrylic chambers were used to create a circulating cigarette smoke environment to the smokers, but no smoke to CG group. The specimens were anaesthetized, sacrificed, and the brains were dissected, frozen and sectioned in a cryostat. Picrossirius-red and hematoxylin-eosin were used for tissue staining, followed by quantification using Motic Images Plus® 2.0 and statistics. The results showed a significant decrease in collagen density in the adventitial tunic of rats exposed to passive smoke as compared to the control for the basilar (CG=59.54%±11.45 and SG=27.89%±7.91; $P<0.05$) and the middle cerebral (CG=55%±9.12 and SG=32.25%±8.35; $P<0.05$) arteries. The thickness of internal elastic lamella of basilar (GC=0.03±0.01/SG=0.01±0.00; $P<0.05$) and middle cerebral (CG= 0.021±0.004 and SG=0.010±0.002; $P<0.05$) arteries indicated a significant decrease in SG as compared to CG. It may be concluded that passive smoking can affect internal elastic lamella and the collagen of the adventitia in both the basilar and the middle cerebral arteries. These alterations corroborate the

effects of nicotine on cerebrovascular weakening associated with inflammatory conditions and degradation of metalloproteinases.

Disclosures: **R.N. Isayama:** None. **E. Robello:** None. **N.L. Froio:** None. **C.A. Fabrega-Carvalho:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.27/M6

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

Support: NIH R00 ES017781

Northeastern University Startup

Title: Loss of DMT1 function is associated with elevated brain copper levels and ADHD-like behavior

Authors: ***M. HAN**, J. CHANG, J. KIM;
Northeastern Univ., Boston, MA

Abstract: Divalent metal transporter 1 (DMT1) is a major iron transporter required for intestinal iron absorption and erythropoiesis. Homozygous Belgrade (b/b) rats display microcytic anemia due to DMT1 mutation. While DMT1 can transport several other divalent metal nutrients, including manganese and zinc, hippocampus-specific DMT1 deficient mice demonstrated impaired prepulse inhibition and cognition, suggesting a novel role of DMT1 in brain metal metabolism and behavioral organizations. To characterize neurobehavioral and neurochemical phenotypes of DMT1 deficiency, b/b rats (12-wk old) and their littermate heterozygous (+/b) rats as controls were subject to elevated plus maze task. After euthanasia, brain tissues were collected to quantify metal levels by inductively coupled plasma mass spectrometry and to determine monoamine-associated protein levels by western blot analysis. In the elevated plus maze test, b/b rats spent more time in open arms (196% increase; $p<0.001$) and less time in closed arms (49% decrease; $p<0.001$) compared with controls, indicating reduced anxiety-like behavior in DMT1 deficiency. In addition, b/b rats entered open arms more frequently (93% increase; $p<0.001$) and traveled more distance in the maze (41% increase; $p=0.017$) than +/b controls. These results suggest increased impulsivity and hyperactivity. The ADHD-like behavior in b/b rats likely results from loss of DMT1 function rather than anemic effect because dietary iron-deficient rats

with normal DMT1 function demonstrated increased anxiety and hypoactivity. Western blot analysis showed up-regulation of dopamine D1 receptors (905% increase; p=0.012) and down-regulation of norepinephrine transporter (28% decrease; p=0.030), vesicular monoamine transporter (35% decrease; p=0.001) and catechol-O-methyl transferase (59% decrease; p=0.044), indicating altered monoaminergic function associated with DMT1 deficiency. Furthermore, metal analysis revealed that b/b rats display reduced brain iron levels, but elevated copper levels in most brain regions, including striatum, cortex and hippocampus. Quantitative PCR data showed that both copper importer Ctr1 (34%; p=0.019) and exporter Atp7a (38%; p=0.022) were up-regulated in the cortex from b/b rats, indicating that brain copper transport is enhanced in DMT1 deficiency. Since brain copper overload is associated with neurological and psychiatric symptoms, our results suggest that copper loading in DMT1 deficiency could impair dopamine metabolism and signaling and promote ADHD-like behavior.

Disclosures: **M. Han:** None. **J. Chang:** None. **J. Kim:** None.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.28/M7

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

Title: Consumption of fluoridated water leads to damage on the prefrontal cortex III layer of the rat

Authors: *P.-V. MARIA ISABEL;
Univ. De Guadalajara, Lagos De Moreno, Mexico

Abstract: Fluoride is a highly toxic substance. Dysfunction of the central nervous system has been considerate as a result of chronic fluorosis. It has been proposed that excessive accumulation of fluoride can exert toxic effects on many tissues and organs, resulting in serious damage and pathological changes. The prefrontal cortex (PFC) is vital region of the brain regulates thought in terms of both short-term and long-term decision making. It allows humans to plan ahead and create strategies, and also to adjust actions or reactions in changing situations. Additionally, the PFC helps to focus thoughts, which enables people to pay attention, learn, and concentrate on goals. This area is also the part of the brain that allows humans to consider several different yet related lines of thinking when learning or evaluating complex concepts or tasks. The effect of fluoridated water consumption on neuronal density and cell damage of III layer of rat PFC was evaluated. Three groups were formed: control (T), Experimental I (EI) and

Experimental II (EII). T and EII groups ingested water jug during pregnancy and lactation of pups until weaning (day 21 of postnatal age), the control group consumed water jug to 130 days, while the pups EII ingested fluoridated water to 4 ppm, from weaning to 130 days. The EI group was exposed to fluoridated water consumption to 4 ppm during the period of pregnancy, lactation and up to 130 days old. After which sacrificed, the brain was removed and PFC were dissected for analysis and counting III neuronal layer. In the case of animals administered fluoride in their drinking water, the increased levels of fluoride were detected in the plasma, in addition, less density and alterations in the forms of neurons were observated like a dark and picnotic cell. The exposure to F can disrupt the synthesis of neurotransmitters and receptors such as acetylcholine (ACh), Cholinergic neurotransmitter are necesary for learning and memory in animals and human. Cholinesterase activity have specific roles in the development of the nervous system such as regulation of neurite outgrowth, proliferation and differentiation of nerve cells. Another way, the exposure to F also produces neural dysplasia and other damage on the synthesis of proteins in the brain, leading to degenerative changes in neurons.

Disclosures: **P. Maria Isabel:** A. Employment/Salary (full or part-time):; Universidad de Guadalajara.

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.29/M8

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

Support: EISAI Inc.

Title: Eribulin and paclitaxel differentially affect mouse sciatic nerve biochemistry: implications for mechanisms underlying chemotherapy-induced peripheral neuropathy

Authors: S. J. BENBOW[1], B. M. COOK[1], K. M. WOZNIAK[2], B. S. SLUSHER[2], B. A. LITTLEFIELD[3], L. WILSON[1], *S. FEINSTEIN[1], M. JORDAN[1];
[1]Neurosci Res. Inst., Neurosci. Res. Inst., Santa Barbara, CA; [2]Neurol., Brain Sci. Inst., Johns Hopkins School of Medicine, Baltimore MD, MD; [3]EISAI Inc., Andover, MA

Abstract: Microtubule targeting agents (MTAs) are widely used as cancer chemotherapies. They prevent spindle formation and induce cell cycle arrest and apoptosis in rapidly dividing tumor cells. Systemic delivery of MTAs often leads to serious side effects, including chemotherapy-induced peripheral neuropathy (CIPN). CIPN symptoms range from numbness/tingling to severe

pain that can be treatment limiting and life threatening. The frequency, severity and reversibility of CIPN vary among different MTAs. As MTAs also vary in their mechanisms of microtubule binding and resulting mechanisms of action, we hypothesize that these different mechanisms underlie the variability seen in CIPN symptoms. To begin testing this hypothesis, we examined biochemical changes in microtubule composition, tubulin post-translational modifications and the levels of select microtubule associated proteins, all of which affect microtubule dynamics and microtubule action. We compared sciatic nerve tissue from mice treated with a Q2Dx3 schedule for 2 weeks at the maximum tolerated doses of paclitaxel or eribulin. Sciatic nerves were fixed, sectioned and immunostained with antibodies directed against α-tubulin, acetylated-tubulin and the microtubule (+) end binding proteins EB1 and EB3. Images were acquired by confocal microscopy and analyzed with Imaris software to quantitate drug-induced changes. Tissue from paclitaxel treated mice exhibited an ~1.9 fold increase in axonal α-tubulin signal, and an ~5 fold increase in acetylated tubulin signal, whereas eribulin treated mice surprisingly exhibited an ~2.5 fold increase in α-tubulin signal, an ~11.7 fold increase in acetylated tubulin signal and an ~2.2 fold increase in EB1 signal. The stronger molecular changes observed with eribulin treatment correlate with less severe axon degeneration in mice and fewer reported instances of severe peripheral neuropathy in humans. We conclude that the increased tubulin, acetylated tubulin and EB1 levels likely indicate increased levels of stable and growing microtubules that could be neuro-protective from toxic drug-induced effects. Subsequent biochemical characterizations in sciatic nerve DRG will provide additional insights into underlying mechanisms of CIPN.

Disclosures: **S.J. Benbow:** A. Employment/Salary (full or part-time):; EISAI Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); EISAI Inc. **B.M. Cook:** A. Employment/Salary (full or part-time):; EISAI Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); EISAI Inc. **K.M. Wozniak:** A. Employment/Salary (full or part-time):; EISAI Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); EISAI Inc. **B.S. Slusher:** A. Employment/Salary (full or part-time):; EISAI 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.; EISAI Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); EISAI Inc.. F. Consulting Fees (e.g., advisory boards); EISAI Inc. **B.A. Littlefield:** A. Employment/Salary (full or part-time):; EISAI 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.; EISAI Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); EISAI Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EISAI Inc. **L. Wilson:** 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; EISAI Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); EISAI Inc. **S. Feinstein:** 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; EISAI Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); EISAI Inc. **M. Jordan:** 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; EISAI Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); EISAI Inc..

Poster

692. Methamphetamine and Drug Induced Toxicity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 692.30/M9

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

Support: Supported by the CounterACT Program, National Institutes of Health Office of the Director (NIH OD), and the National Institute of Neurological disorders and stroke (NINDS), Grant Number 1R21 NS089487

Title: Selective regional targets of hydrogen sulfide poisoning in a mouse model

Authors: *W. RUMBEIHA[1], P. ANANTHARAM[2], B. MAHAMA[2], E. WHITLEY[3], A. KANTHASAMY[2];
[1]VDPAM, [2]Iowa State Univ., Ames, IA; [3]Pathogenesis LLC, Gainesville, FL

Abstract: Hydrogen sulfide (H_2S) is an interesting molecule because in physiologic concentrations it is a gasotransmitter and in toxic concentrations it is neurotoxic. It is endogenously present in the brain playing such essential roles as a neuromodulator, or as an antioxidant, among others. Although its neuronal physiologic mechanisms are not well known, H_2S is believed to effect its function partly through the NMDA type of glutamate receptor. In toxic concentrations, H_2S is believed to increase glutamate release leading to excitotoxicity and neuronal cell death. Currently, there is considerable interest in pharmacological donors of H_2S for therapeutic applications in human brain diseases. This potential therapeutic application of H_2S opens the possibility of H_2S-induced iatrogenic poisoning. The neurotoxicity of H_2S is not well defined, particularly at the molecular level. We have developed an inhalation mouse model of H_2S-induced neurotoxicity. Using this animal model we have demonstrated that H_2S

selectively induces neurodegeneration in the central inferior colliculus (CIC). Acute exposure to 310 ppm H_2S by inhalation for 15-40 minutes significantly increased the glutamate/GABA ratio in the central inferior colliculus. We have also established that H_2S significantly impairs ATP synthesis in the hippocampus. We postulate that the vulnerability of the CIC and the hippocampus are causal to behavioral changes such as motor incoordination and impaired learning and memory we observed following H_2S poisoning in our mouse model. Current ongoing work in our laboratory includes deciphering the molecular pathogenesis of the H_2S-induced neurodegeneration in the CIC. Broadly, this work will improve our understanding of the neurotoxic mechanisms of H_2S which are currently unknown. This knowledge has relevance in clinical settings. Understanding the toxic molecular mechanisms of H_2S-induced neurotoxicity will lead to identification of translational therapeutic targets of H_2S poisoning.

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Poster

693. Somatosensory and Pain Disorders

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 693.01/M10

Topic: C.13. Sensory Disorders

Support: JSPS KAKENHI Grant Number 23249012

Title: LPA-induced peripheral itch sensation and cellular signaling involving LPA_5 receptor, phospholipase D and TRPA1/TRPV1

Authors: *H. KITTAKA[1], K. UCHIDA[1,2], N. FUKUTA[1], M. TOMINAGA[1,2];
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Abstract: The sensation of itch, called pruritus is defined as "an unpleasant cutaneous sensation provoking a desire to scratch". Many pruritogens that are itch-inducing substances have been revealed to activate receptors expressed in dorsal root ganglion (DRG) neurons that initiate itch signaling in the periphery to the central nervous system. Among them, lysophosphatidic acid (LPA) was found as a pruriogen involved in cholestatic itch. On the other hand, LPA was reported to induce acute pain. Therefore these reports have made it unclear whether LPA induces itch, pain or both in the periphery. We first observed behaviors of mice by a cheek injection method and it revealed that LPA caused itch-related scratching behaviors without increasing

pain-related wiping behaviors. In addition to the ambiguity of the LPA-induced mouse behaviors, not only the receptors expressed in DRG neurons involved in LPA action, but also the underlying molecular signaling induced by LPA in DRG neurons have been largely unknown. Therefore, we further examined those mechanisms caused by LPA using a Ca^{2+}-imaging method, a patch-clamp technique and a cheek injection method. LPA induced increases of cytosolic Ca^{2+} concentration in mouse DRG neurons. Itch-related scratching behaviors and Ca^{2+} responses caused by LPA were revealed to be mediated via activation of transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) which are critically important for itch sensation. In addition, pharmacological approaches focusing on LPA receptors and intracellular phospholipases which are expressed in DRG neurons showed that LPA_5 receptor and downstream phospholipases D (PLD) play a crucial role in the LPA-induced Ca^{2+} signaling. We also found that LPA directly induces TRPA1 current from an intracellular side, but not an extracellular side and identified the interaction sites located close to transmembrane domains of TRPA1. This LPA action proposes a cell biological concept that *de novo* intracellular LPA production caused by extracellular LPA leads to activation of TRPA1. Moreover, the itch-related scratching behaviors induced by LPA were decreased by PLD inhibitor treatment, indicating that not only TRPA1 and TRPV1, but also PLD are involved in the LPA signaling *in vivo*. From these results, we concluded that LPA is a pruritogen to induce peripheral itch sensation through the signaling of LPA_5, PLD, TRPA1 and TRPV1 in DRG neurons. Our results provide the detailed LPA-induced itch signaling with the promising mechanism to comprehend cholestatic itch.

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Poster

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Topic: C.13. Sensory Disorders

Support: NIH Grant NS053422

Title: Increased resurgent sodium currents in Nav1.8 contribute to nociceptive sensory neuron hyperexcitability in small fiber neuropathy

Authors: *Y. XIAO, C. BARBOSA, T. CUMMINS, R.;
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Abstract: Small fiber neuropathy (SFN) frequently associated with neuropathic pain is a significant public health challenge. The underlying mechanisms remain poorly understood. Painful SFN may be caused by gain-of-function mutations in Nav1.8, a sodium channel subtype predominantly expressed in peripheral nociceptive neurons. We studied two mutations in Nav1.8 that impair fast inactivation; G1662S reported in painful SFN and T790A reported to cause sensory neuron hyperexcitability in the Possum mutant mouse line. Here we show that in dorsal root ganglion (DRG) neurons, these mutations significantly increase TTX-resistant resurgent sodium currents mediated by Nav1.8, which are atypical sodium currents evoked during the repolarization phase of action potentials. While the G1662S mutation exhibits a one-fold increase compared to wild type Nav1.8, the T790A mutation increases resurgent currents five-fold. We also show that the T790A mutant channels greatly enhance DRG neurons excitability by reducing current threshold and increasing firing frequency. Interestingly, the mutant channels endow DRG neurons with multiple early afterdepolarizations (EADs) at membrane potentials of -20 to -10 mV, leading to almost 20-fold prolongation of action potential duration. In L4-L5 DRG neurons, siRNA knockdown of sodium channel β4 subunits fails to significantly alter T790A current density, but reduces TTX-resistant resurgent currents by 56%. Accordingly, DRG neurons expressing T790A mutant channels exhibit reduced excitability with fewer EADs and shortened action potentials. These results suggest that increased TTX-resistant resurgent sodium currents mediated by Nav1.8 play important roles in EAD generation and action potential prolongation. We propose that increased TTX-resistant resurgent currents by gain-of-function mutations in Nav1.8 may underlie the hyperexcitability of nociceptive sensory neurons that contributes to altered sensory phenotypes, including neuropathic pain in SFN.

Disclosures: **Y. Xiao:** None. **C. Barbosa:** None. **T. Cummins:** None.

Poster

693. Somatosensory and Pain Disorders

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Topic: C.13. Sensory Disorders

Support: NIH Grant 5R01DE221'29-02

Title: A model for herpes zoster opthalmicus

Authors: *C. P. STINSON;
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Abstract Background: Herpes Zoster leads to post-herpetic neuralgia in twenty percent of patients causing chronic pain. In models where varicella is injected into the paw, the virus can induce a nociceptive response for over fifty days. Herpes zoster ophthalmicus occurs when the varicella-zoster virus is reactivated in the ophthalmic division of the trigeminal nerve and occurs in ten to twenty five percent of patients with herpes zoster. Currently, there are no orofacial models for herpes zoster although the disease commonly affects the facial region. Methods: In this study, MeWo cells infected with varicella virus (Poka strain) was injected into the whisker pad of adult male Sprague Dawley rats. The virus was left to incubate and the nociceptive response tested weekly for 8 weeks. The nociceptive response was tested using a thermal contact assay at 45 degrees and von Frey filament orofacial filament test (modified Ugo Basile test chamber). A place escape avoidance paradigm testing the facial region innervated by V2 was also utilized with these animals. Controls included injections of non-infected MeWo or vehicle. Results: A significant increase in mechanical hyperalgesia was observed with the von-Frey filaments and in the place escape avoidance paradigm after injection of virus versus controls. No significant increase in thermal hyperalgesia was measured in the virus injected animals. Conclusion: We conclude that injection of varicella leads to a significant increase in sensory and affective responses in the orofacial region. These results suggest that this varicella injection method can be used as an animal model for orofacial herpes zoster and herpes zoster opthalmicus.

Disclosures: C.P. Stinson: None.

Poster

693. Somatosensory and Pain Disorders

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Topic: C.13. Sensory Disorders

Support: IHS Fellowship award

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Title: Increased EEG gamma-band power and spontaneous cortical spreading depression in Cav2.1 R192Q mice

Authors: *T. HOUBEN[1], R. SHYTI[1], R. R. KLEVER[1], T. J. L. PERENBOOM[1], M. SCHENKE[1], M. D. FERRARI[1], K. EIKERMANN-HAERTER[2], C. AYATA[2], A. M. J. M. VAN DEN MAAGENDBERG[1], E. A. TOLNER[1];

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Abstract: Migraine has been linked to cortical hyperexcitability. The aura of migraine is likely caused by cortical spreading depression (CSD), a slowly spreading wave of neuronal and glial depolarization. Familial hemiplegic migraine 1 (FHM1) mutant mice display enhanced glutamatergic neuronal excitability, as evidenced from cellular recordings, and increased CSD susceptibility in the visual cortex in anesthetized mice. It is unknown whether neuronal hyperexcitability is observed also at the cortical network level. Furthermore, it is not clear whether the enhanced CSD susceptibility in FHM1 mice is dependent on cortical location or time-of-day. In the present study, we performed continuous day-night DC-EEG and multi-unit activity recordings in visual and motor cortex from freely behaving FHM1 R192Q and wild-type (WT) mice. Parallel DC-recordings were performed under anesthesia to compare CSD susceptibility between the start of the light and dark period, as well as between visual and motor cortex. Under freely behaving conditions FHM1 mice displayed enhanced gamma (30-45 Hz) EEG power in both visual and the motor cortex throughout vigilance states, while delta (0.75-5 Hz) power was reduced in motor cortex during REM and non-REM sleep. In 4 out of 10 FHM1 mice, spontaneous CSD events occurred that were never observed in WT mice. CSD events occurred both during light and dark periods. For 9 out of 13 events, the start of CSD was observed first at the visual cortex while for 4 events CSD appeared simultaneously in visual and motor cortex. Under anesthesia, CSD frequency did not show a relation with time-of-day and was enhanced in FHM1 mice compared to WT in both visual and motor cortex. Taken together, the changes in EEG power and occurrence of spontaneous CSD events in FHM1 mutants is in line with increased cortical network excitability. This finding is consistent with enhanced CSD susceptibility in both visual and motor cortex. Cortical network changes and occurrence of spontaneous CSD events in FHM1 mice provide a unique opportunity to study the episodic nature of migraine attacks.

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Poster

693. Somatosensory and Pain Disorders

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Title: STAT3-dependent reactive astrocytes in the spinal dorsal horn contribute to the maintenance of chronic itch in mice

Authors: *M. SHIRATORI-HAYASHI[1,2], K. KOGA[1,2], H. TOZAKI-SAITOH[1,2], Y. KOHRO[2], J. HACHISUKA[3], H. OKANO[4], M. FURUE[3], K. INOUE[2], M. TSUDA[1,2]; [1]Life Innovation, Grad. Sch. of Pharmaceut. Sciences, Kyushu, Fukuoka-shi, Japan; [2]Mol. and Syst. Pharmacol., Grad. Sch. of Pharmaceut. Sci. Kyushu Univ., Fukuoka, Japan; [3]Dermatol., Grad. Sch. of Med. Kyushu Univ., Fukuoka, Japan; [4]Physiol., Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Chronic itch is an intractable symptom of inflammatory skin diseases, such as atopic and contact dermatitis. Recent studies have revealed neuronal pathways selective for itch, but the mechanisms by which itch turns into a pathological chronic state are poorly understood. Using mouse models of atopic and contact dermatitis, we found that astrocytes are markedly activated in the dorsal horn of the spinal segments corresponding to the itchy skin, and remained reactive over a long period. We further found that STAT3 was selectively activated in dorsal astrocytes and that conditional disruption of astrocytic STAT3 activation prevented reactive astrocytes and chronic itch without affecting acute physiological itch. Pharmacological inhibition of spinal STAT3 alleviated fully developed chronic itch. Moreover, atopic dermatitis mice exhibited a striking enhancement of scratching evoked by intrathecal GRP, an itch-inducing neuropeptide, and this phenotype was normalized by suppressing astrocytic STAT3. Therefore, STAT3-mediated reactive astrocytes in the dorsal horn are necessary for chronic itch by inducing spinal sensitization of itch and thus may be a previously unrecognized target for treating chronic itch.

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Poster

693. Somatosensory and Pain Disorders

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Support: CIHR Grant HOP126788

AIHS Graduate Studentship

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Title: Interleukin-1β decreases potassium conductance but does not alter the phenotype of medium sized dorsal root ganglion neurons

Authors: *M.-C. NOH[1,2], P. L. STEMKOWSKI[3], P. A. SMITH[2];
[2]Dept. of Pharmacol., [1]Univ. of Alberta, Edmonton, AB, Canada; [3]Dept. of Physiol. and Pharmacology, Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: There is growing evidence that inflammatory cytokines play an important role in the onset of neuropathic pain. Interleukin 1β has been proposed as one of the important inflammatory cytokines that contributes to the onset of neuropathic pain as IL-1β knockout mice lack neuropathic pain phenotype following a nerve injury. The levels of IL-1β in primary afferents peak at 7d after the nerve injury, and this has been proposed to trigger an enduring alteration in neuronal phenotype that underlies chronic hyper-excitability in sensory nerves to initiate and maintain chronic neuropathic pain. We have shown previously that 5-6 d exposure of rat dorsal root ganglia (DRG) to 100pM IL-1β increases the excitability of medium-sized neurons. We have now found using whole-cell recording that this increased excitability reverts to control levels within 3-4d of cytokine removal. The effects of IL-1β were dominated by changes in K+ currents. Thus, the amplitudes of A-current, delayed rectifier and Ca2+ sensitive K+ currents were reduced by ~68%, ~64% and ~36% respectively. Effects of IL-1β on other cation currents were modest by comparison. Since the changes in cell excitability in the presence of IL-1β is reversible, it is unlikely that direct interaction of IL-1β with DRG neurons initiates an enduring phenotypic shift in their electrophysiological properties following nerve injury. Persistent increases in primary afferent excitability following nerve injury may instead depend on altered K+ channel function and on the continued presence of slightly elevated levels IL-1β and other cytokines.

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Poster

693. Somatosensory and Pain Disorders

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Topic: C.13. Sensory Disorders

Support: KAKENHI24659574

Title: Small-animal neuroimaging analysis of the pain matrix in acute and chronic pain model rats

Authors: *Y. L. CUI[1], H. TOYODA[1,2], E. HAYASHINAKA[1], Y. WADA[1], Y. WATANABE[1]; [1]Imaging Application Group, RIKEN Ctr. for Life Sci. Technologies, Kobe, Japan; [2]Brain Function Imaging, Ctr. for Information and Neural Networks, Osaka, Japan

Abstract: Pain is an unpleasant subjective sensory and emotional experience associated with actual or potential tissue damage, and is usually modified by personal memories, emotion, and cognition. Increasing numbers of non-invasive neuroimaging studies in humans demonstrated that the nociceptive processing involves widely-distributed brain regions, including the somatosensory, insular, cingulate, and prefrontal cortices, and the thalamus. Recently, we have developed a small-animal neuroimaging method combining 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG) PET imaging with statistical parametric mapping (SPM) analysis to evaluate regional brain activity in the rodent central nervous system. In order to investigate whether the nociceptive pathways in chronic pain overlap with physiological "pain matrix" or not, we analyzed the regional brain activity in a neuropathic pain model of rats produced by ligation of the L5/L6 spinal nerves and then compared with those in an acute noxious mechanical stimulus. In the chronic neuropathic pain model, the regional brain activity was significantly increased in the primary somatosensory cortex hind limb area, primary motor cortex, centrolateral thalamic nucleus, and posterior thalamic nucleus in response to mechanical stimuli to the affected hind paw. In contrast, the highest regional brain activity was observed in the posterior cingulate cortex in response to acute noxious mechanical stimuli, whereas no significant brain activity in the corresponding region was observed under chronic neuropathic pain condition. These results indicate that the nociceptive pathways in chronic neuropathic pain may be different from the physiological "pain matrix" associated with acute mechanical pain.

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Poster

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Support: Eemil Aaltonen Foundation (personal grant to JH)

SHOK, SalWe

Academy of Finland

Title: Disrupted functional connectivity of the sensorimotor cortex in complex regional pain syndrome

Authors: *J. HOTTA[1,2,3], J. SAARI[1,2], N. FORSS[1,3], R. HARI[1];
[1]Brain Res. Unit, Dept. of Neurosci. and Biomed. Engin., [2]Aalto NeuroImaging, Aalto Univ., Espoo, Finland; [3]Clin. Neurosciences, Neurol., Univ. of Helsinki and Helsinki Univ. Hosp., Helsinki, Finland

Abstract: Objective Complex regional pain syndrome (CRPS) causes severe limb pain that movements increase. The sensorimotor skills are impaired not only in the painful limb, but CRPS also tends to affect the functions of the contralateral non-painful limb. Sensorimotor cortex of CRPS patients functions abnormally during movements and somatosensory stimulation, but less is known of its functions during rest. In resting state, healthy brains display multiple networks that are characterized by synchronized low-frequency activity in functional magnetic resonance imaging (fMRI). One such network covers the sensorimotor cortices of both hemispheres. In healthy subjects, experimentally-induced sustained pain disconnects the sensorimotor cortex of the painful body part from the resting-state sensorimotor network and engages it with anterior insula. We thus explored the disruptions in the functional connectivity of sensorimotor cortex in CRPS. **Methods** We recorded 3-T fMRI from 12 female CRPS patients who had suffered from right upper-limb pain for 1.4−15.5 years and from 18 healthy female control subjects. Subjects were instructed to rest still and keep their eyes open. Those showing signs of somnolence in online eye-camera monitoring were excluded from the analysis. For the alert subjects (eight patients and fifteen control subjects) we computed seed-based functional connectivity of left- and right-hemisphere hand sensorimotor areas on the basis of fMRI data. **Results** In healthy subjects, the functional connectivity map of each hand area included the whole rolandic sensorimotor cortex of both hemispheres. In patients, the connectivity of the left-hemisphere hand area (corresponding to the painful hand) was statistically significantly decreased, in both hemispheres, with the rolandic cortex medial to the hand areas but increased with the ipsilateral inferior parietal lobule. The right-hemisphere hand area (corresponding to the non-painful hand) displayed similar abnormalities, but its connectivity was strengthened−the more the longer the disease had lasted−with the left and right anterior insulae and the right secondary somatosensory

cortex. **Conclusions** In CRPS patients, suffering from unilateral pain, the resting-state sensorimotor network was altered in both hemispheres, likely reflecting the influence of the continuous pain on brain connectivity.

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Poster

693. Somatosensory and Pain Disorders

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Title: The comparison and new findings of invasive and non-invasive neuromodulation methods

Authors: *R. ROKYTA[1], J. FRICOVA[2];
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Abstract: We are comparing the invasive and non-invasive neuromodulation methods which are used for the treatment of chronic pain.As concern of invasive method we shall compare the peripheral nerve stimulation (PNS), spinal cord stimulation (SCS), deep brain stimulation (DBS) and motor cortex stimulation (MSC).From non-invasive methods we are focused on TENS (trancutaneous nerve stimulation),tDCS (transcranial direct current stimulation),TES (trancranial electric stimulation) and rTMS (repetitive transcranial magnetic stimulation). About novel approaches of transcranial electric stimulation (TACS, TRNS, TRNS) is not known too much. In transcranial alternating current stimulation (TACS) an oscillatory electric current is delivered to the cortical tissue with oscillation frequency between 0.1-5000 Hz. Repetitive TMS (rTMS) has been developed as a diagnostic tool as well. It was observed that the magnetic pulses have a long term neuromodulation effect. This lead to a development of more powerful TMS generators capable of delivery of multiple pulses within short time _ the rTMS. rTMS as a clinical tool is a very gentle, non-invasive method and the demonstration of the success of this treatment is a major step to non-invasive methods of pain therapy. According to recent studies (Rokyta and Fricova 2014), this method is able to induce changes in the central nervous system at the cellular level including changes at ionic and metabolic level. Therapeutic effects rTMS was confirmed in the literature of psychiatric disorders such as depression, acute mania, bipolar disorder, panic attacks, hallucinations, obsessive states, schizophrenia, catatonia or post-traumatic stress disorder

(Rokyta and Fricova, 2012). In patients with Parkinson's disease, dystonia neurological stimulation was neurological stimulation was used as well as in patients with tics, stuttering, tinnitus, seizures or epilepsy, or functional disorders of aphasia after a stroke. rTMS showed prolonged therapeutic effect by reduction or elimination of chronic pain. More recent studies suggest (Fricova et al. 2013) the involvement of the peripheral and central nervous system as a possible mechanism in the pathophysiology of atypical odontalgia. Besides, the pain became a test for the application of electrical stimulation of the brain's motor cortex (MCS) and subsequently it was shown in some cases rTMS had prolonged therapeutic effect including reduction and sometimes even complete elimination of chronic pain. As a rTMS the similar comparison is used also for other above mentioned invasive and non-invasive neuromodulation methods.

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Poster

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Topic: C.13. Sensory Disorders

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Title: Differential modulation of default mode network (DMN) connectivity by self-administered acupressure in fatigued breast cancer survivors

Authors: ***J. HAMPSON**[1], E. ICHESCO[2], V. VERMA[2], B. D. WRIGHT[2], T. KHABIR[2], S. ZICK[2], R. E. HARRIS[2];
[1]Anesthesiol., UNIVERSITY OF MICHIGAN, Ann Arbor, MI; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Introduction: Breast cancer (BC) survivors often present with a cluster of symptoms including persistent fatigue, pain, and depression. While the factors generating the cluster are currently unknown, central neurobiological dysfunction is thought to be involved. Recently we demonstrated that fatigued breast cancer survivors displayed increased functional connectivity of the Default Mode Network (DMN) to the frontal gyrus when compared to non-fatigued cancer controls. We also have shown that self-administered acupressure can alleviate symptoms of fatigue in breast cancer survivors. Here we sought to explore the neurobiological underpinnings of self-administered acupressure techniques on resting state network (RSN) connectivity in

breast cancer survivors. Methods: Nineteen BC survivors with persistent fatigue were randomized to receive either relaxation (RA: n=9) or stimulation (SA: n=10) acupressure over the course of 6 weeks. All participants underwent resting state fMRI at baseline and following treatment. Group independent component analysis was done using GIFT and validated using ICASSO software. SPM group level analysis was performed to compare change in RSN connectivity across each treatment group using a two-sample t-test. Change in clinical pain (BPI), depression (HADS) and fatigue (BFI) were individually correlated to change in RSN connectivity using within group covariate of interest analyses. A two-way ANOVA analysis was also performed to study interaction between brain connectivity between groups and clinical symptoms. Significant clusters were identified at thresholds $P<0.05$ FDR/FWE cluster correction. Results: Following treatment the DMN showed greater connectivity to the bilateral thalamus ($P=0.02$) in the SA group and increased connectivity to the periaqueductal gray ($P=0.005$ SVC) in the RA group. Within the RA group change in pain severity was positively correlated to change in connectivity between DMN and posterior cingulate ($P=0.01$) and mid-temporal gyrus ($P=0.05$). In contrast, a negative correlation was found between change in depression and DMN to perigenual cingulate connectivity ($P=0.05$) in the SA group. Two-way ANOVA analysis showed differential relationships between treatment groups: DMN to mid-temporal gyrus showed positive correlation to pain (r=0.87) for RA and negative relationship (r=-0.30) for SA ($P=0.04$). Conclusion: Our findings suggest that self-administered acupressure formulas have differential effects on symptoms in in BC survivors. These effects were associated with modulation in connectivity to the DMN. It is unclear if similar findings would also be observed in other cancer conditions.

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Poster

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Topic: C.13. Sensory Disorders

Title: Human sensory neurons derived from iPS as a human *in vitro* model of peripheral pain

Authors: *E. GRAS LAVIGNE[1], D. BUTTIGIEG[2], L. L'HOMME[2], C. BADJA[3], M. OUAMER[2], R. STEINSCHNEIDER[2], F. MAGDINIER[3];

[1]Neuronexperts, Marseille, France; [2]Neuron Experts, Marseille, France; [3]INSERM UMR_U910 GMGF, Marseille, France

Abstract: Somatosensory disorders due to pathological causes (peripheral neuropathies), to spinal impairment or to injured peripheral nerves can generate a wide range of symptoms. Peripheral pain, one of the most prevalent symptoms, can be unbearable for patients. As the sensitivity of a specific receptor varies between species, a model based on human sensory neurons seems to be of interest. First, we aimed to influence/improve peripheral pain by the enhancement of peripheral nerves regeneration capacity. Some compounds by their positive trophic effect could positively influence the growth of sensory neurons and re-innervate skin by wound healing or injured nerves restoration. To discriminate compounds according to their trophic effect, we developed a relevant *in vitro* cellular model of peripheral pain based on human sensory neurons derived from human iPS cells. We first formed spheroids of neuron precursors to incubate them in the presence of compounds, previously trapped to each pole of the culture well. We validated our cellular model by using NGF as reference compound and demonstrated a preferential growth of neurites network toward NGF. We finally developed a monolayer culture of neuron precursors differentiated in sensory neurons in a 96 well plate model in order to screen compounds according to their trophic actions on the neurite length. The second studied parameter of peripheral pain was the resulting activation of mechanoreceptors and nociceptors. To separately study each sensory receptor, we used human sensory neurons cultured in 96 well cell plates. We characterized several sensory receptors by immunostaining. This model allowed us to carry out/implement several functional studies based on the ability of sensory neurons to release neurotransmitters after specific stimulations in order to screen compounds according to their capacity to decrease nociceptors activation. The technology of human fibroblast differentiation into human sensory neuron enabled to build in-vitro models of compounds screening with predictable results comparable to human in-vivo studies results and is of great interest for further studies.

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Poster

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Title: Function and dysfunction of Kv3.4 channels in DRG neurons: implications in pain signaling and peripheral pain sensitization induced by SCI

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Abstract: Kv3.4 channels underlie a majority of the high-voltage activating K+ current in dorsal root ganglion (DRG) nociceptors, and are involved in the modulation of action potentials and spiking in the DRG. Additionally, Kv3.4 channel downregulation is implicated in neuropathic pain induced by spinal cord injury (SCI). It remains to be seen whether specific Kv3.4 channel manipulations can influence nociceptor excitability and pain signaling. Previous work also demonstrated that phosphorylation of the Kv3.4 N-terminal inactivation domain at multiple sites slows inactivation kinetics, and that mutations at these sites dramatically alter the Kv3.4 current profile. To investigate more directly the impact of the Kv3.4 channel on pain signaling, we have tested the functional expression of recombinant mutants in acutely dissociated DRG neurons. We introduced the mutants by nucleofection and used cell-attached patch-clamp electrophysiology to examine their functional expression. The results show that Kv3.4-S(8,9,15,21)A (phosphorylation-resistant) and Kv3.4-S(8,9,15,21)D (phospho-mimetic) induce robust currents larger than the endogenous currents from mock nucleofected neurons (5-fold and 6-fold, respectively). Whereas S(8,9,15,21)A currents exhibit a fast inactivating profile, the S(8,9,15,21)D mainly show slow or no inactivation. Therefore, these mutants will be useful tools to test the role of Kv3.4 channels in different "phosphorylation states" on the excitability of DRG neurons (*in vitro*) and pain signaling (*in vivo*). We are currently 1) creating AAV viruses to knock-down Kv3.4 and express mutants *in vivo*, and 2) implementing methodology to examine synaptic transmission in the dorsal horn.

Disclosures: T. Muqeem: None. **V. Pinto:** None. **M. Covarrubias:** None.

Poster

693. Somatosensory and Pain Disorders

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Topic: C.13. Sensory Disorders

Support: 1.W81XWH-11-1-0672. US Department of Defense, Grant# 10669042

Title: Intravenous injection of mesenchymal stem cells reversed morphine tolerance in mice

Authors: *J. CHENG, L. LIU, Z. HUA, H. WU;
Pain Mgmt. and Neurosciences, Cleveland Clin., Cleveland, OH

Abstract: Backgrounds: Opioid therapy is commonly used for the relief of moderate to severe pain, including cancer pain and various chronic pain conditions. However, chronic or repeated administration of opioids often results in the development of opioid tolerance and thus limits its efficacy and safety. **M**esenchymal **S**tem **C**ells (MSCs) are multipotent cells that possess powerful anti-inflammatory effects. One of the proposed mechanisms for opioid tolerance is that opioids such as morphine can directly activate microglia and cause release of proinflammatory cytokines. We have demonstrated that intrathecal transplantation of MSCs significantly attenuated opioid tolerance induced by daily morphine injections. In this study, we tested if intravenous administration of MSCs can reduce opioid tolerance induced by daily morphine injections in mice. **Methods:** With IACUC approval, mice were treated with morphine (10mg/kg, s.c.) or normal saline once a day for 14 days. A single intravenous injection of MSCs derived from rat bone marrow was administered on day 15. Opioid tolerance was assessed by the difference in latencies of tail flick measured before and 30 minutes after morphine injections for 13 days. A large difference indicates low tolerance and a small difference indicates high tolerance. **Results**: Consistent reduction of tail flick latency was observed with repeated morphine injections, compared to the pre-injection control. This is consistent with the concept of opioid-induced hyperalgesia. Opioid tolerance developed in Day 8 of daily morphine injections in both male and female mice in control group, as reflected by the reduced differences in tail flick latencies measured before and after the morphine injection. Intravenous injection of MSCs significantly reversed this effect of repeated morphine injections in both male and female mice. Opioid-induced hyperalgesia was also attenuated, but only in male mice. There was no difference in body weight between the transplantation and control groups. **Conclusions**: Our observations suggest that intravenous injection of MSCs is a promising therapy to treat morphine-induced opioid tolerance. There may be a gender difference in responsiveness to MSCs therapy, which need to be further investigated. The cellular and molecular basis of MSC therapy is being actively studied in our lab.

Disclosures: J. Cheng: None. **L. Liu:** None. **Z. Hua:** None. **H. Wu:** None.

Poster

693. Somatosensory and Pain Disorders

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Topic: C.13. Sensory Disorders

Title: The effect of visual information produced by modified mirror therapy in patients with phantom limb pain

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Abstract: Introduction We administered mirror therapy to patients with phantom limb pain. Although the therapeutic principle of this therapy remains to be elucidated, it is hypothesized that mirror therapy enables patients to obtain the sensation of being able to move their phantom limb voluntarily and reduces their pain. To help patients better image the mirror limb, we established a new method “modified mirror therapy (mMT)”, wherein, we recorded a video of the movement of the patient's unaffected limb and reversed the image and corresponded it to the phantom limb. In this study, we evaluated the effect of mMT in healthy volunteers and patients with phantom limb pain by investigating the function of primary motor cortex using functional near-infrared spectroscopy (fNIRS). Method All experimental protocols were approved by the ethics committee. Study 1: Three healthy volunteers were enrolled. We recorded videos of their right hand movement (grasped and opened) and evaluated the activity of the brain cortex during this movement by analyzing changes in oxygenated hemoglobin (oxyHb) levels by using fNIRS (FOIRE3000: Shimadzu). The channels covered the left and right primary motor cortex across the central zone. For fNIRS examination, movements were recorded for 10 s. The volunteers performed the following movements: 1. Movement of the right hand 2. Imaginary movement while watching the video Study 2: Six patients with phantom limb pain were enrolled. The patients performed the following movements: 1. Imaginary movement of the phantom limb 2. The mMT: Imaginary movement of the phantom limb while watching the reversed video of their unaffected limb 3. Imaginary movement of the phantom limb without the video after mMT for 10 min Result Study 1: A marked increase in oxyHb was mainly confined to primary motor cortex corresponding to the right arm during movement. While watching the video of right hand movement, a marked increase in oxyHb was also mainly confined to the primary motor cortex. Study 2: Imaginary movement of the phantom limb increased oxyHb over a wide area, including the primary motor cortex. mMT increased oxyHb in the primary motor cortex region corresponding to the phantom limb. An increase in oxyHb became confined to the primary motor cortex during imaginary movement of the phantom limb without the video after performing mMT for 10 min. The phantom limb pain reduced or disappeared within 1 month of repeated mirror therapy in all patients. Conclusion We were able to evaluate the influence of visual information in the primary motor area by using fNIRS. mMT may lead to functional reorganization of the primary motor cortex in patients with phantom limb pain.

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Poster

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Topic: C.13. Sensory Disorders

Support: CNRS (contract UPR3212)

Université de Strasbourg

Title: Phosphodiesterases as targets for the treatment of neuropathic pain

Authors: ***I. YALCIN-CHRISTMANN**, S. MEGAT, V. LELIEVRE, A. LACAUD, E. WALTISPERGER, R. SCHLICHTER, M.-J. FREUND MERCIER, S. HUGEL, M. BARROT; CNRS-DR10 INCI UPR3212, Strasbourg cedex, France

Abstract: Antidepressants are a first-line treatment against neuropathic pain. Noradrenaline recruited by their action on reuptake transporters has been proposed to act through beta2-adrenoceptors (beta2-ARs) to lead to the antiallodynic effect. However, the precise downstream mechanism remains to be identified. It is well known that beta2-AR stimulation increases cyclic adenosine-monophosphate production, which is controlled by phosphodiesterase (PDEs). The aim of the present study was to study therapeutic potential of PDE inhibitors (PDEi) and further identify underlying mechanisms by using a neuropathic pain model in mice. Neuropathy was induced by implanting a unilateral cuff around the main branch of the sciatic nerve of C57BL/6J mice. This procedure induces unilateral sustained mechanical allodynia, measured with von Frey filaments. By using pharmacological approaches, we showed that the PDE4i could alleviate mechanical allodynia after chronic treatment. Indeed, PDE4 inhibition decreased expression of Tumor necrosis factor (TNF)alpha in dorsal root ganglia of neuropathic animals and this antiallodynic mechanism also depends on the recruitment of delta opioid receptors. We also showed that the action of a PDE4i is dose dependent since these drugs activate glial cells at low doses and neurons at high doses. In conclusion, our results show the therapeutic potential of PDE inhibitors in the treatment of neuropathic pain.

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Poster

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Support: ErasmusMC grant

Stichting Erasmus MC Pijnfonds

Title: Bortezomib-induced peripheral neuropathy: clinical data and nerve fiber density measurements in skin biopsies

Authors: M. BECHAKRA, *J. L. JONGEN;
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Abstract: Bortezomib-induced peripheral neuropathy (BiPN) is a frequent and disabling complication in patients treated for hematological malignancies. Early recognition is of utmost importance, to prevent irreversible neurological damage and persistent pain. This is the first study that systematically evaluates clinical data and nerve fiber densities in lower leg skin biopsies of 22 patients. Symptoms and signs from the neurological examination, Sensory Sum-Score (SSS), Common Toxicity Criteria of Adverse Events (CTCAE), orthostatic hypotension, Numerical Rating Scale (NRS) of pain intensity, McGill Pain Questionnaire (MPQ) and Neuropathic Pain Scale (NPS) were recorded in these patients. Also, intraepidermal nerve fiber densities (IENFD) of PGP9.5-labeled nerve fibers were determined and compared with age-matched healthy controls. Finally, correlations between pain scores and IENFD were studied. Mean±SD SSS and CTCAE for sensory neuropathy and/or pain in our patients were 6.8±3.2 and 1.18±1.0 respectively, indicating light-moderate sensory neuropathy. Orthostatic hypotension and pain intensity >4 were present in 62% and 77% of the patients, indicating small nerve fiber involvement in the majority of cases. Mean±SD pain intensity NRS and overall MPQ were 5.4±0.7 and 21.0±9.7 respectively, indicating moderate neuropathic pain. IENFDs however, were not statistically significantly different from healthy volunteers (5.5±2.0/mm and 5.7±2.3/mm respectively). Although a statistically significant correlation between the worst pain ($r=0.46$; $p<0.05$) and deep pain ($r=0.44$; $p<0.05$) items of the MPQ and NPS respectively was found, the correlation between the other MPQ and NPS items, pain intensity NRS, overall MPQ and IENFD was not statistically significant. We conclude that BiPN is a sensory neuropathy with substantial small nerve fiber involvement. The finding that IENFDs in BiPN patients were not statistically significantly different from healthy volunteers may indicate relatively mild BiPN in

our cohort. The finding that no correlation between pain intensity NRS, overall MPQ and IENFD was found may also reflect relatively mild BiPN in our cohort or that neuropathic pain is driven by selective degeneration of only a subset of intraepidermal nerve fibers.

Disclosures: M. Bechakra: None. **J.L. Jongen:** None.

Poster

693. Somatosensory and Pain Disorders

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Support: 1R01 GM102525

R21 DA0344480

Harold Carron Professorship

Title: 5β- reduced neurosteroid enhances recovery and reduces hyperalgesia in a rodent model of postoperative pain

Authors: *S. JOKSIMOVIC[1], D. F. COVEY[2], K. KRISHNAN[2], V. JEVTOVIC-TODOROVIC[1], S. M. TODOROVIC[1];
[1]Anesthesiol., Univ. of Virginia/Old Med. Sch., Charlottesville, VA; [2]Washington Univ. Sch. Med., St.Louis, MO

Abstract: **Introduction** Neuroactive steroids are potent modulators of neuronal activity in the peripheral and central nervous system causing anesthetic, analgesic and anticonvulsant effects. It has been shown that synthetic 5β-reduced steroid analogues exhibit a potent local analgesic effect *in vivo*. The aim of our study was to investigate spinal and local analgesic potency of 3β-OH ((3β,5β,17β)-3-hydroxyandrostane-17-carbonitrile), a 5β-reduced steroid, in a model of acute postoperative pain in rats. **Methods** To assess antinociceptive efficacy in an acute postoperative pain model using deep tissue incision, thermal stimulation of the plantar surface of Sprague Dawley rat hind paws was performed and paw withdrawal latency (PWL) was measured. ***Results*** We have found that intrathecal injection of 3β- OH induced dose- dependent thermal antinociception in naïve rats in comparison to the vehicle- injected group with maximal effect 60 minutes after injection (maximal dose 16 μg per 50 μl, n=8- 11). Single intrathecal application of the highest effective dose from previous experiments (16 μg) injected after incision produced significant acute antihyperalgesic effect 24 and 48 h post- surgery comparing to vehicle group

(n=6- 13, measured during 180 minutes). Similarly, repeated i.t. application of the same dose in the same timeframes in a time- dependent manner increased PWLs of incised paw 24 and 48 h post- surgery, but not 2 h post- surgery (n=6- 13, measured during 180 minutes). Single intraplantar application (16 or 48 μg per paw) of 3β- OH in incised paw did not exert acute antihyperalgesia in thermal nociception (measured 60 minutes post-injection). However it had a long- lasting antihyperalgesic effect as evidenced by increased baseline PWLs measured daily during post- operative days 1, 2 and 3, in comparison to the vehicle group (n=6). Repeated intraplantar application 2, 24 and 48 h after surgery significantly increased baseline PWLs of the incised paw during 1- 5 days of post- incision follow up, in comparison to vehicle- injected group (dose 16 μg per paw, n=6-8). ***Conclusion*** These results suggest that 3β- OH could be used spinally to reduce thermal nociception in healthy rats. Similarly, post-surgical single or repeated spinal application could alleviate thermal nociception after surgery. Furthermore, even though single intraplantar application did not produce acute antinociceptive effect in incised animals, it did increase the baseline PWL during follow up period of 3 days. As a similar effect was noticed after repeated intraplantar application, these data indicate that local peripheral application of 3β-OH could decrease recovery time after surgery in rats.

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Poster

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Title: Activation of protein kinase B mediates pain related responses induced by the bone metastases of breast cancer cells

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Abstract: The Protein kinase B, also called Akt, is known to regulate cell proliferation and growth by controlling protein translation. Recently, it has been shown that Akt signaling pathway is involved in long-term synaptic plasticity. However, the role of Akt under different pain conditions, especially in cancer pain, is less clear. In this study, the dynamic activation of Akt in dorsal root ganglia (DRG) which contributes to pain responses induced by bone metastases of breast-cancer cell was investigated. In this study, the left tibia metastases of breast-cancer cell line Wlker-256 was found to induce time-dependent thermal and mechanical hyperalgesia. Simultaneously, the activation of Akt, as well as its downstream molecule mammalian target of rapamycin (mTOR), was found in rat ipsilateral L5-L6 DRG neurons, which started from day 7 and peaked at day 21 after the cancer cell implanted. In addition, intrathecal (i.t.) injection of GSK690693, a pan inhibitor of Akt, was observed to reduce pain related behavioral responses. Thus, these results indicate that Akt signaling pathway is involved in the hypersensitivity induced by bone metastases of breast-cancer.

Disclosures: **F. Jiang:** None. **G. Ding:** None.

Poster

693. Somatosensory and Pain Disorders

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Support: Functional MRI Laboratory of the University of Michigan PG U034199

Title: Altered resting state connectivity in individuals with fibromyalgia following an acute pain stimulus

Authors: *E. ICHESCO[1], T. PUIU[2], J. P. HAMPSON[2], A. E. KAIRYS[3], D. J. CLAUW[2], S. E. HARTE[2], S. J. PELTIER[2], R. E. HARRIS[2], T. SCHMIDT-WILCKE[4];
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Abstract: INTRODUCTION: Fibromyalgia (FM) is a chronic widespread pain condition, with patients commonly reporting other symptoms such as sleep difficulties, memory complaints, and fatigue. The use of magnetic resonance imaging (MRI) in FM has allowed for the detection of neural abnormalities in this condition, with augmented brain activation elicited by experimental pain and alterations in resting state connectivity related to clinical pain. Here, we sought to

monitor state changes in resting brain connectivity following experimental pressure pain inFM patients and healthy controls (HC). METHODS: 12 female FM patients and 15 age-matched HC were studied by applying individually adjusted pressure stimuli (equal pain intensity) to the left thumbnail bed during MRI. Resting state functional MRI scanning was performed before and immediately following experimental pressure pain. We investigated changes in functional connectivity to the thalamus and the insular cortex with a seed based region of interest analysis using the Functional Connectivity Toolbox (www.nitric.org/projects/conn) ¬ in SPM8. Subject-specific connectivity maps for each seed were entered into a second-level repeated measures ANOVA (two-factorial design) with group and time point as factors. Analyses included a 1) main effect of group at baseline and post pain, 2) main effect of time within each group, and 3) a group by time interaction. Results were deemed significant with a family wise error (FWE) cluster-level corrected P value < 0.05 derived from a voxel-wise uncorrected P value < 0.001. Connectivity maps from significant results were correlated with clinical symptoms in SPSS v.21. RESULTS: Acute pressure pain increased insula connectivity (analysis 1) to the anterior cingulate ($z = 4.71$, ¬P¬ = 0.015) and the hippocampus ($z = 4.01$, $P = 0.036$) moreso in FM patients than HC. We observed increased thalamic connectivity to the precuneus/posterior cingulate cortex ($z = 4.81$, $P = 0.019$), a known part of the Default Mode Network, in FM but not HC (analysis 3). Increased thalamic connectivity to the cingulate/precuneus was also associated with increased clinical pain ($r = 0.610$, $P = 0.046$). CONCLUSION: These data suggest that an acute painful stimulus may alter the neural signature of chronic pain. It is unknown if these findings are generalizable to other chronic pain conditions.

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Poster

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Title: Music-induced analgesia increases the amplitude of BOLD fMRI in the left angular gyrus

Authors: *E. A. GARZA-VILLARREAL[1,2], P. VUUST[2], F. A. BARRIOS[4], L. VASE[3], E. BRATTICO[5], E. PASAYE-ALCARAZ[4], T. S. JENSEN[6], Z. JIANG[7];
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Abstract: Fibromyalgia (FM) is a chronic pain disease without cure, where patients can present moderate to intense generalized (and sometimes incapacitating) pain (Wolfe, 2013). Pleasant and relaxing music reduces pain importantly in FM by unknown mechanisms. To study the fMRI representation of analgesia with music, we performed an analysis technique that measures the amplitude of low frequency fluctuations (0.01 - 0.08 Hz or DMN) in rsfMRI called fALFF. We hypothesized that music-induced analgesia would elicit activity in areas of the pain modulation system. Twenty fibromyalgia female patients participated in the study. Inclusion criteria included diagnosis of FM of > 1 year, right handedness and moderate to intense spontaneous pain. During the study, their spontaneous pain was measured using a Verbal Rating Scale (0 - 10). We scanned the patients using a GE Discovery MR750 3T scanner using resting state BOLD gradient echo sequences (TR = 3000 ms, TE = 40 ms, flip angle = 90, matrix = 128 x 128, FOV = 256 mm, slice thickness = 3 mm). The patients listened to experimenter chosen noise and self-chosen music (high valence, low arousal). Before and after each auditory stimulus we performed pain ratings and rsfMRI. The data were preprocessed using the fALFF technique with the AFNI software and we performed statistical analysis using FSL software (paired t-test, FWE correction). Finally, we extracted the time series data from the resulting fALFF cluster to perform correlations with the pain ratings using the Δ (delta) values (Cpos - Mpos). The FM patients reported significantly less pain after listening to music. In the fMRI contrast Music > Noise, we found a significant cluster of higher amplitude in the left angulary gyrus (x=-38, y=-68, z=36, $p < .001$, mean-T = 5.05, size = 64 voxels). We also found a significant negative

correlation between the Δ fALFF Z-score and Δ pain intensity Z-score (r = -.56, p = .03), meaning the report of analgesia was correlated to an increased amplitude of low frequency fluctuations in the left angular gyrus. In this study we found that listening to self-chosen pleasant and relaxing music reduced pain in fibromyalgia patients, and the analgesic effect was correlated to high amplitude of low-frequency fluctuations in the left angular gyrus, an important brain region of the default-mode network also found in pain modulation. In this study, the mechanism behind music-induced analgesia in FM patients seems to be top-down, elicited probably by distraction, emotion and reappraisal. This is the first study of its kind and should be replicated with other forms of cognitive analgesia in fibromyalgia.

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Poster

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Title: Cortical spreading depression induced by targeted optogenetic activation of cortical pyramidal neurons

Authors: *Z. KILLEEN, A. PARGA, J. NICHOLS, C. WU, T. ANDERSON;
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Abstract: Migraine is one of the most common neurological disorders characterized by the recurrence of severe debilitating headaches. While the underlying cause of migraine remains unknown many patients report an “aura” that precedes their migraine pain and is often perceived as sensory disturbances. Cortical spreading depression (CSD) is a slow moving wave of depolarization that propagates across the cortex and is the pathophysiological mechanism behind migraine aura. Intrinsic optical signal (IOS) imaging has proven a powerful technique to monitor and track the development of CSD. Experimental CSD induction paradigms have traditionally used mechanical stimulation (“pin-prick”), high potassium solutions or osmotic disruption. While successful in their induction of CSD these methods are often invasive and have provided limited ability to target activation of specific neuronal populations. To overcome this we herein describe a novel method of combining IOS imaging with simultaneous photostimulation of

channelrhodopsin (hChR2). Cortical pyramidal neurons were selectively targeted by restricting hChR2 expression using stereotaxic injection of adeno-associated virus (AAV) expressing the hChR2 gene under the CAMKIIa promoter or transgenically expressed using cre-lox technology. Activation of hChR2 expressing neurons in cortical brain slices with blue light (470nM) reliably induced CSD that was of similar in phenotype to more traditional high potassium methods. Overall, these data suggest that targeted activation of cortical pyramidal neurons is sufficient to induce CSD. Furthermore, that optogenetic approaches are compliant with traditional imaging methods and provide a valuable approach to study CSD and migraine pathophysiology.

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Poster

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VIEP through project in the area of health in favor of JREC and MCCS

Title: Study of the nociceptive response in female high yawning rats with an anxious trait

Authors: *S. L. RUGERIO[1], J. R. EGUIBAR[1,2], C. CORTES[1];
[1]Inst. de Fisiología, [2]Res. Office-VIEP, Benemérita Univ. Autónoma De Puebla, Puebla, Mexico

Abstract: The high- and low- yawning (HY and LY, respectively) differ one order of magnitude in their spontaneous yawning frequency. The HY and LY rats also differ in the number of grooming bouts in a novel environment or after wetting the fur and in the open-field arena suggesting that HY rats are more anxious. Chronic pain, stress, and anxiety have been considered a public health problem, since 20% of the population suffers them and these illnesses affect in greater proportion to women with 3 to 1 female/male ratio. The aim of this study is to evaluate pain threshold responses along estrous cycle in HY and LY female rats using the tail-flick latency apparatus, as well as the effects of ovariectomy (OVX) and estrogen administration We

studied 32 adult female rats. We measured tail-flick latencies in five control sessions, three additional measurements after OVX and three after subcutaneous 20 μg estradiol benzoate (EB) during 15 days. Our results showed that pain threshold were higher in the LY subline (4.325 s latency), after OVX both sublines showed similar tail-flick latencies and EB administration induced higher effects in LY than HY rats. We concluded that HY and LY sublines differ in analgesic responses and are suitable model for the study of the relationship between sex steroid hormones and pain threshold and probably chronic pain.

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Poster

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Title: Nociceptive reflex stimulus intensity threshold or reflex amplitude? A painful choice in the objective quantification of central sensitization

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Abstract: Central Sensitization (CS) is a maladaptive response of neurons within the central nervous system to persistent peripheral nociceptive input. While CS has been linked to an increasing number of clinical conditions, there is currently no reliable clinical technique for quantifying changes in CS. The nociceptive reflex is commonly employed to assess changes in spinal excitability (CS). The stimulus intensity threshold (SIT) required to elicit a reflex response and the nociceptive reflex amplitude are two common outcome measures used interchangeably to assess spinal excitability; however, the responsiveness of these two outcomes to changes in spinal excitability has yet to be directly compared. The purpose of this study was to compare the effect of experimentally induced CS on the nociceptive reflex SIT to the nociceptive reflex amplitude in young healthy adults. We tested the primary hypothesis that the nociceptive reflex amplitude will increase and nociceptive reflex SIT will decrease following experimentally induced CS. Our secondary hypothesis states that a strong inverse correlation ($r>0.7$) exists between changes in the nociceptive reflex SIT and nociceptive reflex amplitude after the experimental induction of CS. Twelve (12) young healthy adults (23.1±2.74) participated in this crossover-design study. Electrical noxious stimuli (five, 1ms square wave pulses) were delivered

every 10 to 15 seconds to the right index finger at 2mA increments from detection threshold while reflex responses were recorded from upper limb muscles (deltoid, biceps brachii, lateral triceps, flexor carpi ulnaris, extensor carpi radialis) via surface electromyography. Baseline nociceptive reflex responses were recorded prior to the induction of CS. CS was evoked in the C5 neuromere by the topical application of capsaicin cream (Zostrix 0.075%) along the lateral forearm (50cm^2) compared to placebo cream applied on a different session separated by 1 week. Nociceptive reflex responses were recorded at 10, 20, 30, and 40 minutes post-sensitization. The reflex SIT was significantly reduced following experimentally induced CS ($p<0.05$) while the reflex amplitude showed no significant increase following experimentally induced CS ($p>0.05$). The relationship between reflex amplitude and reflex SIT demonstrated a strong inverse relationship in the placebo condition ($r=-0.901$); however, this relationship was significantly weaker following the induction of CS ($r=0.507$). These observations suggest that reflex amplitude and reflex SIT may represent different underlying physiologic mechanisms and may not be interchangeably used to assess changes in central sensitization.

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Poster

693. Somatosensory and Pain Disorders

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Title: Sepiapterin reductase as a new target to reduce chronic pain hypersensitivity

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Abstract: Most putative new analgesic treatments developed from targets identified in pre-clinical studies have failed in phase 2 trials, possibly because of species differences. To increase to chances of translational success we selected a pathway with clinical validation, identified by genome-wide association studies. GTP Cyclohydrolase 1 (GCH1) polymorphisms which decrease tetrahydrobiopterin (BH4) levels are associated with reduced pain in patients in various chronic pain conditions. In this study we tested if the BH4 production pathway was relevant in chronic pain sensitivity and if targeting this pathway could be viable therapeutic approach. Using mice that express eGFP under the GCH1 promoter we show that excessive BH4 is produced by both axotomized sensory neurons and macrophages infiltrating damaged nerves and inflamed tissue. We find that constitutive BH4 overproduction in sensory neurons is sufficient to increase pain sensitivity, whereas blocking BH4 production only in these cells using tissue-specific inducible GCH1 KO mice prevents or reduces nerve injury-induced hypersensitivity without affecting nociceptive pain. To minimize risk of precipitating side effects by substantially decreasing global BH4 levels we targeted sepiapterin reductase (SPR), whose blockade allows low level BH4 production through sepiapterin processing in the BH4 salvage pathway. We used a structure-based design to develop a potent SPR inhibitor that allows systemic administration with no adverse effects and characterized sepiapterin accumulation as a sensitive biomarker for SPR inhibition *in vivo*. SPR inhibition significantly reduced pain hypersensitivity in two models of peripheral nerve injury (spared nerve injury and chronic constriction injury) with a concomitant decrease in BH4 levels in injured sciatic nerve and dorsal root ganglia, with no development of tolerance. This treatment was also effective to reduce hyperalgesia in two models of chronic inflammatory pain (intraplantar or knee injection of Complete Freund's Adjuvant), most likely by inhibiting macrophages' function. Altogether our results show that SPR represents a good target for developing novel analgesic treatments for neuropathic and inflammatory pain.

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Poster

693. Somatosensory and Pain Disorders

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Title: Suppression of oral cancer and chemotherapy-induced chronic pain symptoms by a synthetic peripherally-restricted cannabinoid receptor agonist

Authors: Y. MULPURI[1], D. DANG[2], B. L. SCHMIDT[2], H. H. SELTZMAN[3], *I. SPIGELMAN[1];

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Abstract: Oral cancer pain and chemotherapy induced peripheral neuropathy (CIPN) are significant public health problems. Current medications for pain alleviation of these diseases often lack efficacy and exhibit undesirable side effects. Preclinical studies have demonstrated the analgesic effectiveness of brain-penetrant cannabinoids (CB) in the treatment of cancer and CIPN pain symptoms, but their use may be limited by the psychotropic side effects of central CB1 receptor (CB1R) activation. We recently succeeded in the development of several novel CB1R agonists that do not appreciably cross the blood-brain barrier and which suppressed pain symptoms induced by sciatic nerve injury without central side effects (Mulpuri et al, SFN Abstr. Vol. 37:173.21, 2012). Here we examined one of these compounds (4-{2-[-(1E)-1[(4-propylnaphthalen-1-yl)methylidene]-1H-inden-3-yl]ethyl}morpholine, PrNMI) for effectiveness in alleviating pain symptoms in mouse oral cancer models and in a rat model of CIPN. Oral cancer-induced mechanical allodynia was suppressed after systemic PrNMI administration. In these mice, we also observed a >40% reduction in tumor volume. Paw cancer (inoculated with human oral carcinoma cells) mechanical allodynia was also dose-dependently suppressed by systemic PrNMI. Oral administration of PrNMI dose-dependently suppressed cisplatin (3 mg/kg, 1/week for 4 wks)-induced mechanical and cold allodynia with complete symptom suppression at 3 mg/kg. Daily oral administration at 1 mg/kg consecutively for two weeks resulted in similar daily suppression of mechanical and cold allodynia implicating little, if any, tolerance development. Intraplantar PrNMI (0.25 mg/kg) injection completely suppressed CIPN symptoms, suggesting peripheral sensory nerve terminals as the main sites of PrNMI's anti-allodynic action. PrNMI co-administration with selective CB1R or CB2R blockers revealed mainly CB1R contribution to its analgesic effects. In the central side effects assays, brain-penetrant HU-210 exhibited potent side effects at systemic doses that relieve neuropathy symptoms, whereas PrNMI and its analogs showed a complete lack of side effects in tests for catalepsy, hypothermia and motor incoordination. Similar results were obtained with female rats. The potency, peripheral selectivity, *in vivo* efficacy, and absence of CNS side effects of this novel class of CBR agonists point to their potential as a viable treatment for cancer and CIPN pain symptoms.

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Poster

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Banting Postdoctoral Fellowships program

Title: Neuropathic pain promotes gene expression adaptations in stress and depression related brain regions

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Abstract: Neuropathic pain is a complex condition characterized by sensory symptoms such as allodynia, hyperalgesia and dysesthesia. Around 65% of neuropathic pain patients also develop depression. A growing amount of clinical and preclinical studies reveal that neuropathic pain conditions lead to changes in neuronal activity in several brain networks involved in mood and motivation, including the Nucleus Accumbens (NAc) and the medial prefrontal cortex (mPFC). In this study, we employed the spared nerve injury model (SNI) of neuropathic pain (or control sham surgery) in adult C57Bl\6 mice and performed RNA-sequencing, in order to monitor changes in gene expression in three brain regions known to be implicated in the pathophysiology of depression as well as in the modulation of neuropathic pain symptoms: the NAc, the mPFC, and the periaqueductal grey (PAG). Two months post-surgery, SNI mice displayed mechanical allodynia as well as increased depression-like behaviors. Mice were sacrificed two and a half months post-injury, and RNA samples were extracted and assessed through Illumina based next generation deep RNA-sequencing. Although some common gene expression changes were observed, unique transcriptional profiles were identified across the three brain regions. Real-time PCR and western blot analyses were used to validate gene expression patterns and to further characterize the pathways involved in neuropathic pain symptoms. This is the first unbiased characterization of neuropathic pain induced long-term gene expression changes in three distinct brain regions composed of predominantly different neuronal populations (glutamatergic, GABAergic, and serotonergic).

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Poster

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Support: 2014m2c1b2048632

Title: The therapeutic potential of neurokinin 1 receptor antagonist for arthritic pain and cartilage destruction in rat model of osteoarthritis

Authors: T. KIM[1,2], H. JEON[1,2], Y. KIM[3], E. SONG[1,2], Y. YOON[3], *J. KIM[1,2];
[1]Korea Univ. Coll Hlth. Sci., Seoul, Korea, Republic of; [2]Dept. of Publ. Hlth. Sci., Rehabil. Sci. Program, Korea Univ., Grad. Sch., Seoul, Korea, Republic of; [3]Korea Univ. Col. Med., Seoul, Korea, Republic of

Abstract: Osteoarthritis (OA) is the most common progressively degenerative disease occurring in the elderly. The factors related to the mechanism of the osteoarthritis are neuropeptides, cytokine, growth factors, and protease. Among these, Substance P (SP) is the neuropeptides, which released from the nociceptive primary afferent fiber and is responsible for neurogenic inflammation and pain transmission through neurokinin 1 (NK1) receptor activation. Thus, this study investigated the therapeutic potential of local injected an NK1 receptor antagonist on neurogenic inflammation in OA. Under anesthesia with 2% isoflurane, 4 mg of monosodium iodoacetate (MIA) was intra-articularly injected into the right knee in male Spraque-Dawley rats (200-220 gram). GR 82334 (10 uM), NK1 antagonist, was intra-articular administrated 30 min before (pre-treatment group) or 1 day after MIA injection (post-treatment group). After drug injection in MIA induced OA rats, knee joint diameter, knee bending test, paw withdrawal threshold to mechanical stimuli (Up down method using von Frey filaments) and weight bearing were assessed. Joint structures and SP immunoreactivity were also analyzed in the knee joint. In both pre and post treatment of GR82334 group, the knee joint diameter was decreased at 2 days, and paw withdrawal threshold to mechanical stimuli was elevated at 10 days after MIA injection. However, weight bearing and the knee bending test was no different. Histological degenerative change of knee joint structures and synovial SP and CGRP immunoreactivity were less presented. Thus, NK1 antagonist may regulate the inflammation and degeneration of knee joint structures that holds the possibility of secondary hyperalgesia alleviation in osteoarthritis.

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Poster

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Title: Peripheral nerve injury alters synaptic input and intrinsic connectivity in lamina II excitatory dorsal horn neurons in neuropathic pain states

Authors: *N. GONG[1], T. IKRAR[2], G. HAGOPIAN[1], Z. LUO[1], X. XU[2];
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Abstract: The spinal dorsal horn is the first central relay area for pain transmission and modulation. While previous anatomical and electrophysiological studies examined spinal dorsal horn circuitries, functional studies of circuit connections and network activity are required to further understand spinal cord sensory information processing in acute and chronic pain states. Our previous studies suggested that peripheral nerve injury enhanced presynaptic excitatory input onto spinal superficial dorsal neurons, which contributed to nociception development. However, the detailed local postsynaptic circuits underlying heightened behavioral sensitivity in the dorsal horn remain largely unexplored. We combined whole cell electrophysiological recordings with laser scanning photostimulation (LSPS) to test whether spinal nerve ligation (SNL) led to enhanced excitatory synaptic input and/or reduced inhibitory input to lamina II excitatory dorsal horn neurons. Td-tomato-fluorescence labeled, vesicular glutamate transporter 2 (Vglut2)-expressing excitatory neurons were recorded from dorsal horn lamina II in living slice preparations of mouse L4 lumbar slices. High-resolution LSPS was applied to map glutamate-evoked excitability of and local synaptic connectivity to the recorded lamina II excitatory neurons. We analyzed and compared the data recorded from ipsilateral (injured) versus contralateral (control) sides of the lumbar slices 1-2 weeks after the nerve injury when injured mice showed pain hypersensitivity. We found that SNL did not significantly affect intrinsic membrane excitability of superficial dorsal horn excitatory neurons. However, SNL (1-2 weeks) increased glutamate-evoked excitability, shown as increased photostimulation-evoked spiking sites as well as total evoked spikes from recorded dorsal horn neurons in the injury side, averaged 2.1 and 3.7 times of the control side, respectively. Concurrently, SNL enhanced local excitatory synaptic circuit connections to superficial dorsal horn excitatory neurons. The average

total excitatory synaptic inputs of recorded neurons in the injury side were about 5.7 times that of the control side. Local inhibitory synaptic circuit connections to the recorded superficial dorsal horn neurons were decreased 1-2 weeks after SNL. The average total inhibitory synaptic inputs were 17.0% of the control. Together, these data support that SNL-induced maladaptive changes in synaptic input/connectivity to superficial dorsal horn excitatory neurons, which provides new insight in neural circuit basis of pain hypersensitivity.

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Poster

693. Somatosensory and Pain Disorders

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Topic: D.08. Pain

Support: This project was supported by NIH (1R01DE022129-01A1) Grant.

Title: Lateralization of pain-related neural oscillations in the central nucleus of the amygdala

Authors: ***M. KAJUMBA**, A. L. HARRIS, J. N. STRAND, Y. PENG;
Psychology Dept., Univ. of Texas At Arlington, Arlington, TX

Abstract: The evidence for lateralization of pain-related changes in the CeA has mainly emerged from *in vitro*, and a few *in vivo* studies using anesthetized animals. These procedures eliminate the perceptual component as well as the emotional dimension of pain, and thus the results may not be a complete reflection of the pain experience. Both the left and right CeA receive, and are activated by nociceptive input, but the changes in synaptic plasticity and sensitization have been found to be lateralized in the right CeA. The main goal of this study was to use freely moving animals to determine the differential responses in the left and right CeA activity during the progression of inflammatory pain. Adult Sprague-Dowley rats with: 1) right CeA lesions; 2) left CeA lesions; and 3) no lesions (control), had a bipolar electrode chronically implanted into either the left or right CeA. Inflammatory pain was induced by subcutaneous injection of 2 mg of carrageenan (100µl of 2% (w/v) in saline) into either the left or right hind paw. Local field potential (LFP) oscillations were recorded at 6 time points; that is, baseline, immediately, 1, 3, 5, and 48 -72 hours after the carrageenan injection. The results revealed that: 1) the overall changes in the left CeA oscillations were mainly due to contralateral, while the right CeA oscillations changed in response to both contralateral and ipsilateral injury; 2) there

was initial increase in the delta oscillations (1-4 Hz) of both CeA, but the effect remained in the right, but not left CeA by 48 - 72 hours; 3) alpha oscillations (8-13 Hz) significantly decreased in the right, but not left CeA; 4) the beta oscillations (14-30 Hz) initially decreased in both CeA, but the effect remained significant in the left, but not right CeA by 48 - 72 hours after the carrageenan injection; 5) the left CeA gamma oscillations (30-100 Hz) significantly increased immediately, and at 48 - 72 hours after the ipsilateral injection, and persistently decreased in response to the contralateral injury, while the right CeA oscillations only decreased at 5 hours after the contralateral injection. This is the first *in vivo* study to demonstrate lateralization of pain-related changes in the neural oscillations of the CeA, and provides numerous implications, and insight into directions for future research.

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Poster

694. Fear and Anxiety: Behavior

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

Title: Trait vulnerability to anxiety and chronic stress interact to affect acoustic startle amplitude

Authors: ***M. L. JACOBSON**, P. PEDULLA, D. J. KIM, B. J. ANDERSON;
Psychology and Integrative Neurosci., Stony Brook Univ., Stony Brook, NY

Abstract: Pre-existing individual differences, such as trait anxiety, may make some individuals more susceptible to develop psychopathology than others. To understand the interaction between individual differences and experience, we designed a rodent living environment that allows manipulation of threat stress. Animals were tested on the elevated plus maze (EPM) and then classified as low anxiety (LA) or high anxiety (HA) based on median split of the anxiety index. Animals were then assigned to the control or threat groups with equal numbers of LA and HA in each group. Rats were housed in cages with food and water separated by a tunnel for 3 weeks. When the threat group reached the center of the tunnel, simultaneous presentations of predator odor, abrupt light and sound were presented randomly with a probability of 0.25. The control group crossed without threat presentations. After the 3 week treatment conditions, animals were tested on a battery of behavioral tests. Repeated threat affected acoustic startle response, spatial memory, anxiety index in the EPM (only when tested in aversive test conditions), but not sucrose preference. Individual differences prior to the treatment manipulation did not predict sucrose preference, enhanced spatial memory tested in aversive test conditions, or the anxiety index in an

aversive EPM. However, individual differences were related to acoustic startle. Startle was measured across 2 days, with 2 sessions per day. Individual differences were apparent in the first startle session of each day, but less evident by the second session of each day, when previous threat exposure led to sensitization, whereas the control group habituated. Threat exposed HA animals sensitized to the startle stimuli, whereas control HA animals tended to habituate. A region of the brain implicated in EPM behaviors and startle modulation is the lateral septum (LS). Threat exposure increases metabolic capacity in the LS, hypothalamus and PAG, an axis that supports contextual modulation of innate responses. It is possible that individual differences in LS explain the predictive power of EPM behavior for startle amplitude in session 1. We are currently assessing whether individual differences interact with threat experience to influence metabolic capacity in the LS. In summary, pre-existing individual differences in anxiety measured in the EPM predicted hyperarousal in the first startle session, whereas threat experience had a greater effect on adaptation of the startle response. These studies may aid in identifying the neural substrates that underlie individual differences in susceptibility to stress-related disorders and hyperarousal in PTSD.

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Poster

694. Fear and Anxiety: Behavior

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Program#/Poster#: 694.02/M40

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

Title: Impacts of maternal separation on an animal model of posttraumatic stress disorder using a shuttle box in rats

Authors: ***M. TANICHI**, H. TODA, S. ENOMOTO, K. SHIMIZU, M. UENOYAMA, Y. MASUDA, R. NAKAGAWA, M. NIBUYA, A. YOSHINO;
Natl. Def. Med. Col., Saitama, Japan

Abstract: OBJECTIVES: We have previously reported posttraumatic stress disorder (PTSD) model of rat using a shuttle box, in which rats exposed to inescapable footshocks (IS) corresponding to trauma 2 weeks before had the persistent behavioral alterations characterized by ‘bi-directional changes’. Past clinical studies have shown that exposure to stress during the postnatal development periods is associated with an increased risk for PTSD. In this study, we examined the influence of maternal separation (MS), which is one of the most commonly used procedures for inducing the early life stress in rodents, on our PTSD model. METHODS: Timed

pregnant Wistar rats were delivered on gestation day 14. On postnatal day (PND) 2, litter was assigned to MS or typical animal facility rearing (AFR) groups. MS took place for 3 hours per day for 2 weeks from PND 2 and only male pups were used for the subsequent experiment. On 7 weeks old, the IS session corresponding to trauma was performed, and 2 weeks after the IS session, the avoidance/escape task trials were done. Detailed experimental procedures were described as previously (Sawamura et al, 2004). RESULTS: In the avoidance/escape task trial, the number of the behavior counted as an error, which did not move to the other chamber during the footshock was significantly increased in MS/IS(+) group (13.00 ± 5,45), compared with the other three groups: AFR/IS(-) group (1.00 ± 1.00), AFR/IS(+) group (1.29 ± 0.97), MS/IS(-) group (1.25 ± 0.59). However, MS did not significantly affect the behavioral alterations characterized by 'bi-directional changes', which is related to the symptoms of PTSD. CONCLUSIONS: While the error behavior in the shuttle box system indicate learned helplessness as a depression model of rats, this study may suggests that IS in adolescence subsequent to MS induced the depressive behaviors, not but affect the PTSD symptoms. REFERENCES: Sawamura et al. (2004) Effect of paroxetine on a model of posttraumatic stress disorder in rats. Neuroscience Letters, 357: 37-40.

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Poster

694. Fear and Anxiety: Behavior

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Topic: F.03. Motivation and Emotion

Title: The pain of others: empathy's influence on anxiety- and depression-like behaviour in female mice

Authors: *S. MALDONADO BOUCHARD, A. KLEIN, J. S. MOGIL;
McGill Univ., Montreal, QC, Canada

Abstract: The aim of this study is to identify the behavioural and physiological effects of long-term exposure to a familiar conspecific experiencing chronic pain. The anxiety- and depression-like behaviour and pain behaviour of mice housed in dyads in which either both, one, or neither have undergone spared nerve injury (SNI) were compared. Adult CD-1 mice (7-13 weeks old), female and male, were used. Experimental housing conditions included both female and male

same-sex pairs: SNI + SNI, sham + SNI, and sham + sham. At arrival, the mice were housed two per cage. No earlier than one week following arrival, baseline behavioural measures were collected. Two weeks following arrival, subjects underwent SNI or sham surgery. Standard tests of anxiety- and depression-like behaviour were conducted with all subjects 2 weeks following surgery, and then at 3-week intervals until the end of the experiment, 6 months post-surgery. Anxiety-like behaviour was assessed by examining center time in an open field, and time spent in the open arms of an elevated-plus maze. To assess depression-like behaviour, sucrose preference, and immobility in the forced swim test were used. In addition, tactile allodynia and spontaneous pain were assessed with von Frey filaments (up-down method) and the Mouse Grimace Scale, respectively. Female (but not male) shams housed with an SNI conspecific showed decreased center time in an open field up to 22 weeks after surgery relative to baseline, whereas shams housed with a sham conspecific did not. No differences were found on measures of sucrose preference or tactile sensitivity across housing conditions. Forced swim test performance and physiological correlates of anxiety and depression-like behaviour will be collected at the end of the experiment. Serum will be collected to examine levels of corticosterone as well as cytokines and chemokine via ELISAs. The current data suggest long-term exposure to a conspecific experiencing chronic pain differentially affects females, resulting in increased anxiety-like signs. In humans, providing long-term care for a relative suffering from a chronic illness, including chronic pain, is associated with depression and anxiety. Beyond the consciously perceived burden and psychological stress experienced by caregivers, the chronic exposure to a relative in pain may contribute to decreased well-being by pre-cognitive mechanisms. The next step will be to identify the mechanism via which exposure to a conspecific experiencing chronic pain modulates behaviour.

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Poster

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

Support: NSERC

Title: Examining associative and non-associative fear memories in the rat exposure test

Authors: *K. FALLON, P. MACCALLUM, J. WHITEMAN, T. KENNY, D. SKINNER, G. MARTIN, J. BLUNDELL;
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Abstract: A single exposure to a traumatic psychological event may result in the onset of stress-related disorders such as post-traumatic stress disorder (PTSD). PTSD is characterized by four core symptoms including re-experiencing the traumatic event, avoiding trauma-associated stimuli, hyperarousal, and negative cognition or mood. PTSD can be characterized as a disorder involving disturbed emotional learning and memory processes resulting in enhanced fear acquisition and maintenance. Fear memories can occur in response to environments that were previously associated with the stressor (associative fear memories) or can be apparent in environments different from the stressor, which suggests that these changes are not conditioned, but rather are measures of non-associative fear. Associative memory is often assessed using the classical fear conditioning paradigm whereby an animal learns to associate a novel context (or cue) with an aversive stimulus (i.e., mild foot shock). Upon re-exposure to the context or cue associated with the aversive stimulus, rodents will show species-typical fear behaviors, typically measured as freezing. In contrast, stress-induced changes in hyperarousal (as measured as response to acoustic startle) and anxiety-like behaviors (as measured in the elevated plus maze (EPM), open field (OF) and dark/light box (DL)) are measures of non-associative fear. While many studies have examined the neurobiology of associative fear memories using classical fear conditioning paradigms, few have examined non-associative fear. Thus, the aim of the current study was to develop an animal model of PTSD that produced both associative and non-associative fear. We found that a brief exposure of a mouse to a rat produces both associative and non-associative fear memories. Specifically, a single 5-minute protected exposure of a mouse to a rat causes increased freezing to the predator stress context, increased startle response, and increased anxiety-like behavior in the EPM, OF, and DL box. In contrast, exposure to an acute foot shock produces associative fear, but does not alter non-associative fear memories. These data suggest the mechanism underlying shock-induced and predator stress-induced fear memories are different. Current studies in the lab are examining extinction of predator stress-induced associative and non-associative fear memories. Identification of the neural mechanisms underlying associative and non-associative fear memories processes may aid in understanding the development and/or treatment of PTSD.

Disclosures: K. Fallon: None. **P. MacCallum:** None. **J. Whiteman:** None. **T. Kenny:** None. **D. Skinner:** None. **G. Martin:** None. **J. Blundell:** None.

Poster

694. Fear and Anxiety: Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 694.05/M43

Topic: F.03. Motivation and Emotion

Support: NIA

UW-Research Growth Initiative

Title: Aging-related changes in the neural circuits underlying fear memories

Authors: *M. SEHGAL, T. S. BULA, B. HUMMER, B. K. FULLEYLOVE-KRAUSE, K. L. FELDMANN, J. R. MOYER, Jr.;
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Abstract: Aging leads to gradual changes in neuronal functioning that can be detected as early as middle age. These changes are also evident as impairments in various forms of learning. The current experiments were designed to address two salient aspects of aging related cognitive decline. First, most behaviors impaired with aging require successful interaction between a distributed network of brain structures. Second, although impaired, aging animals can often acquire these tasks albeit more slowly. In order to assess how the brain adapts in the face of these gradual changes to display successful learning is unknown. Specifically, what are the precise changes in the neural circuitry that allow successful learning during aging? Few, if any, studies have investigated how normal aging alters learning-related changes within a neural circuit. To address these issues, we used trace fear conditioning and extinction as a model. Our lab, as well as other labs, previously demonstrated that acquisition of trace fear conditioning, and its extinction, is impaired during normal aging. To investigate circuit-level changes, we quantified immediate early gene (IEG) expression in various brain regions implicated in fear learning. Briefly, young, middle aged, and aged rats were randomly divided into four groups: naïve, unpaired, trace fear conditioned (TRACE) or extinction (EXT). On day 1, TRACE and EXT groups received 10 CS-US trials. The unpaired group received explicitly unpaired CS and US presentations. On days 2 and 3, EXT and unpaired rats received 10 CS-alone trials. On day 3, fear memory was tested in TRACE, EXT, and unpaired rats using 2 CS-alone presentations after which their brains were removed and processed for immunohistochemistry to quantify the expression of Zif-268 and c-Fos. Results from adult rats demonstrate that conditioning as well as extinction increased the number of Zif-268 and c-Fos labeled neurons within retrosplenial cortex as well as the infralimbic and prelimbic subregions of medial prefrontal cortex (mPFC). Interestingly, these robust learning-related changes in adult rats were either absent or reduced in aging rats (both middle aged as well as aged). Moreover, despite differences in IEG expression, the aging rats were able to acquire trace fear conditioning and extinction. Therefore, fear learning engages mPFC and retrosplenial cortex in the adult but not the older rats. These data suggest that the neural circuitry underlying retrieval of fear memories is altered during normal aging. This is

likely to have profound implications in development and targeting of neurotherapeutic strategies for improving cognitive function in aged animals, including humans.

Disclosures: **M. Sehgal:** None. **T.S. Bula:** None. **B. Hummer:** None. **B.K. Fulleylove-Krause:** None. **K.L. Feldmann:** None. **J.R. Moyer:** None.

Poster

694. Fear and Anxiety: Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 694.06/M44

Topic: F.03. Motivation and Emotion

Title: Maternal inhibition of vicarious blood-injection-injury phobic syncope: a case report

Authors: ***M. ONEILL**[1], A. R. HIRSCH[2];
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Abstract: Introduction: Blood injection injury (BII) phobic syncope first described in 1916 effects 15% of the population. While venipuncture is the most common (44%), vicarious stimuli occur in 10%. Familial inhibition of vicarious precipitation has not heretofore been described. Case: Case report: A 42 year-old married female describes, since the age of 18, vasovagal syncope upon the sight of blood. Her first episode of fainting was associated with a venipuncture and occurs whenever exposed to blood. True blood must be present (special effects blood in cinema or theater has no effect). Seeing others bleed, including her husband or siblings, readily induces syncope. However, when confronted with her seven-year-old son's bloody injuries on two separate occasions, she was able to actively participate in his care, without presyncopal symptoms or syncope. General physical, neurologic and psychiatric examinations were abnormal for mildly impaired immediate and remote memory, and poor calculations. Discussion: Possible mechanisms for selective BII phobic syncope include evolutionary advantage to maximize survival of the progeny, predominance of fear/sympathetic response over disgust/parasympathetic discharge, and preferential epinephrine action on α1 over β2 receptors. Conclusion: Selective maternal protection against BII induced syncope suggests that psychological factors can modulate this cardiovascular event. Possibly, artificially inducing fear may act to prevent blood exposure induced syncope. During venipuncture, encouraging a continued fearful state until the disgust dissipates may prevent fainting. Investigation of the therapeutic use of fear in preventing BII phobic syncope is warranted.

Disclosures: **M. Oneill:** None. **A.R. Hirsch:** None.

Poster

694. Fear and Anxiety: Behavior

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Topic: F.03. Motivation and Emotion

Support: Grant-in-Aid for Scientific Research on Innovative Areas

StrategicResearch Program for Brain Sciences by the Ministry of Education, Culture,Sports, Scienceand Technology of Japan

Grant-in-Aid for Scientific Research (B)

Title: The appetitive and aversive context modulates choice behavior, autonomic responses, and the neuronal activity in the primate caudate

Authors: *Y. UEDA, K. NAKAMURA;
Kansai Med. Univ., Osaka, Japan

Abstract: Decision making involves not only the valuation of specific events but also the motivational context such as the net expectation of appetitive and aversive outcomes. The neuronal substrate of the latter process, however, has not been well studied partly due to the lack of objective measures of emotional context. Here we designed a behavioral paradigm in which animals made choice under different emotional context, assessed by the fixation behavior and the autonomic responses. In the task, three fractal images were separately associated with a juice (R), a neutral tone (T), or an aversive airpuff (A). After fixating on a central fixation point (FP) for 1000 ms, a pair of two images, R-T, R-A, or T-A, appeared in the left and right of the FP. The monkeys chose one of the images by eye movements to obtain a reward and/or to avoid a punishment. The same image pair was repeated up to 30 trials as a block while the side of the images changed randomly, which allowed the animal to predict the specific pair before its presentation. In all three monkeys tested (two Macaca fascicularis and one Macaca mulatta), suboptimum choices and fixation variability increased for the pairs that included 'A': R-A (even though another counterpart was 'R') and T-A, than that without 'A': R-T. In two animals whose autonomic responses were measured, the heart rate was significantly higher and pupil size was larger for the pairs that included 'A' than that without 'A'. The changes in the heart rate and pupil size partly predicted the following choice behavior. These findings suggest that the emotional context affects autonomic responses in parallel with the decision making process. We next examined neuronal activity of the caudate, an input channel of the basal ganglia in two monkeys during the performance of the task. Among 207 task-related neurons, 47% (n=97) exhibited

differential modulation in the activity during the fixation period depending on the block type, suggesting that the neurons encoded different value context. Among them, 37 neurons showed stronger activity for the pairs with 'R' (R-T, R-A) than the pair without 'R' (T-A), consistent with the hypothesis that the striatum is involved in reward-based decision making. However, we also found that 53 neurons showed stronger activity for the pairs with 'A' (R-A, T-A) than the pair without 'A' (R-T), indicating the striatum's involvement in encoding aversive context. The results suggest that different subsets of primate caudate neurons encode information about appetitive or aversive context, which may be the neuronal substrate of decision making in different emotional context.

Disclosures: Y. Ueda: None. **K. Nakamura:** None.

Poster

694. Fear and Anxiety: Behavior

Location: Hall A

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Program#/Poster#: 694.08/M46

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

Support: Hokuriku Bank Grant for Young Researchers

Title: Quantitative analysis of monkey emotional gestures by a markerless 3D motion capture

Authors: R. V. BRETAS, T. NAKAMURA, *J. MATSUMOTO, Y. TAKAMURA, E. HORI, T. ONO, H. NISHIJO;
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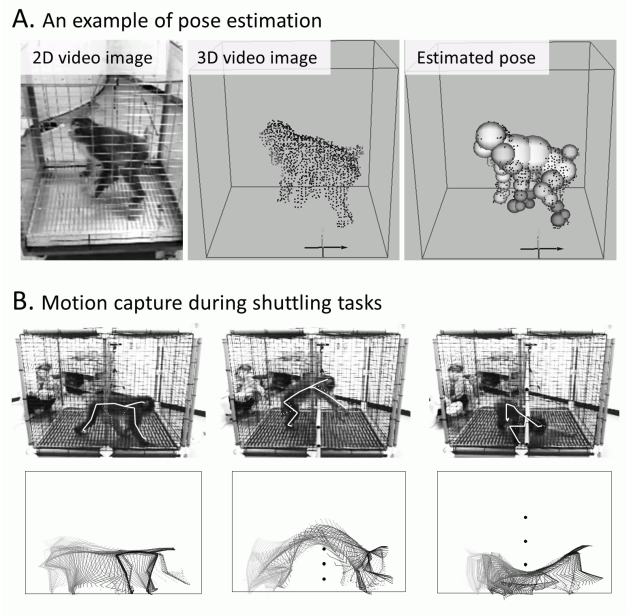
Abstract: Monkeys have similar kinematics to those of humans, enabling skillful forelimb control and standing on hind legs. Motion of body parts and gestures in animals reflect various emotions and intentions as well as ongoing goals. Thus, motion capture in monkeys is an important tool to investigate neural mechanisms of socio-emotional behaviors as well as motor control, which may be inherited by humans. For motion capture, many previous studies used markers attached to various parts of the body. However, those markers could disturb normal behaviors and induce stressful behaviors such as biting, scratching or smelling the markers. In this study, we developed a markerless motion capture system for monkeys (Fig. A) and analyzed behaviors of freely moving monkeys. The 3D positions of the body parts estimated by the 3D system were compared with those estimated by experimenters, using the sample data when a monkey was performing a shuttling behavior to get over an obstacle (Fig. B). The averaged errors of the estimated positions of the body parts were less than 10 cm, suggesting the present

system is useful for analyzing arbitrary monkey movements. Then, we analyzed monkey behavioral responses to a fake snake and a neutral object using this system. The results revealed that a significant increase in clinging behavior duration during presentation of the fake snake (56 ± 6 %) compared with the neutral object (34 ± 5 %; $p < 0.05$), indicating that the monkey avoided the snake. Second, we analyzed behaviors after methamphetamine (MTP) administration (1.0 mg/kg, i.m.) in four monkeys. MTP significantly increased head movements (MTP: 5.7±0.2 m/min; saline: 4.6±0.4 m/min; $p < 0.05$), and decreased locomotion speed (MTP: 8.8±2.0 cm/sec; saline: 17.1±2.8 cm/sec; $p < 0.1$). The results are consistent with a previous study reporting that humans feel jittery after MTP administration. Taken together, these results demonstrate that the present system allows analyses of monkey's emotional motion and gestures in a highly reproducible way.



Disclosures: **R.V. Bretas:** None. **T. Nakamura:** None. **J. Matsumoto:** None. **Y. Takamura:** None. **E. Hori:** None. **T. Ono:** None. **H. Nishijo:** None.

Poster

694. Fear and Anxiety: Behavior

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Topic: F.03. Motivation and Emotion

Support: NIGMS Grant R25GM060566

Title: Intracerebroventricular infusion of neuropeptide Y reduces the behavioral effects of social defeat stress and promotes anxiolytic-like behaviors in Syrian hamsters

Authors: *C. M. MARKHAM[1], T. LACEY[3], K. KENNIEL[3], R. KINGSTON[2], M. EDWARDS[1];
[1]Psychology, [2]Biol., Morehouse Col., Atlanta, GA; [3]Psychology, Spelman Col., Atlanta, GA

Abstract: Our lab utilizes an ethologically relevant model of social stress whereby defeated Syrian hamsters exhibit long lasting changes in behaviors, including increased submissiveness and a complete lack of territorial aggression, even when paired with a smaller, non-aggressive intruder (NAI). A small subset of hamsters, however, appears to be resilient to the effects of social defeat stress. Recent studies have suggested that neuropeptide Y, a widely distributed, 36-amino acid peptide may function to reduce the effects of traumatic stress in a variety of animal models. The aim of this study was two-fold: to determine if intracerebroventricular (icv) infusion of NPY can reduce the behavioral response to social defeat stress in Syrian hamsters and to examine whether NPY has anxiolytic-like properties in the elevated plus maze. The data indicate that icv infusion of NPY prior to social defeat significantly reduced submissive behaviors during testing with a NAI 24-hours later (duration of submission in vehicle group: 94.4 secs +39.5; 400pmol NPY group: 8.9 secs +3.9). In addition, hamsters that were infused with NPY 5 minutes prior to testing in the elevated plus maze showed a significant increase in open arm time (duration in vehicle group: 37.1 secs +12.1; 400pmol NPY group: 165.7 secs + 18.7). These results provide further support for the hypothesis that NPY functions as a resilience factor and is also among the first studies to demonstrate an anxiolytic-like effect of NPY in Syrian hamsters. Studies are currently underway to determine the receptor subtype involved in these effects.

Disclosures: C.M. Markham: None. **T. Lacey:** None. **K. Kenniel:** None. **R. Kingston:** None. **M. Edwards:** None.

Poster

694. Fear and Anxiety: Behavior

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Program#/Poster#: 694.10/M48

Topic: F.03. Motivation and Emotion

Support: NIH MH38774

Title: Norepinephrine transmission between locus coeruleus and central amygdala regulates aversive Pavlovian-to-instrumental transfer

Authors: *V. CAMPESE[1], J. SOROETA[3], E. VAZEY[4], G. ASTON-JONES[4], J. LEDOUX[2], R. SEARS[5];
[1]Ctr. For Neural Sci., [2]New York Univ., New York, NY; [3]Univ. of Arkansas, Fayettville, AR; [4]Rutgers, New Brunswick, NJ; [5]Nathan Kline Inst., Orangeburg, NY

Abstract: Three experiments provide evidence that norepinephrine (NE) transmission in the central amygdala (CeA) modulates Pavlovian-to-instrumental transfer (PIT). Specifically, experiment 1 demonstrated that, compared to vehicle infusions, muscimol infusions into the CeA significantly impaired PIT, confirming the role of CeA in this phenomenon. Experiment 2 found that systemic NE agonism with procaterol eliminated PIT and NE blockade with propranolol increased PIT. Using excitatory designer receptors exclusively activated by designer drugs (DREADDs) experiment 3 found that excitation of neurons in the locus coeruleus (LC) significantly impaired PIT. Generally less responding and weaker transfer were seen in these rats following treatment with clozapine-n-oxide (CNO) compared to vehicle treatment. This was also true when targeting the terminals of DREADD expressing LC neurons in the CeA with intracranial CNO infusions. Ongoing studies are evaluating the effects of LC inhibition with DREADDs on PIT. These studies provide strong evidence that NE transmission in CeA constrains PIT, which is in agreement with findings that implicate NE processes in the CeA as critical to the expression of aversive Pavlovian threat conditioning.

Disclosures: V. Campese: None. **J. Soroeta:** None. **E. Vazey:** None. **G. Aston-Jones:** None. **J. LeDoux:** None. **R. Sears:** None.

Poster

694. Fear and Anxiety: Behavior

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Topic: F.03. Motivation and Emotion

Support: NARSAD Young Investigator Award from The Brain & Behavior Research Foundation

Title: Selectively-bred anxious rat phenotype is characterized by an early emergence of classical fear conditioning

Authors: *D.-J. CHANG[1], J. HIDER[1], P. BLANDINO[1], R. M. SULLIVAN[2], H. AKIL[1], J. DEBIEC[1];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Emotional Brain Inst., NewYork, NY

Abstract: Anxiety disorders are the most prevalent psychiatric disorders in childhood affecting around 10% of youth (Kessler et al., 2005). Maladaptive anxiety emerges early in life and disturbs child's psychosocial functioning and development. It is generally accepted that anxiety disorders are caused by a combination of inborn/hereditary and environmental factors. One of the best studied inborn risk factors for anxiety disorders in youth is anxious temperament whereas one of the best known environmental risk factors for anxiety disorders is a history of emotional trauma in childhood. Fear conditioning (FC) is the most commonly used experimental model of emotional trauma. In FC a neutral stimulus (conditioned stimulus, CS), such as neutral sound or odor is paired with an aversive unconditioned stimulus (US), typically a mild electric shock. As a result, upon subsequent exposures to the CS, an organism expresses threat behaviors, such as freezing. Previous rodent studies show that fear conditioning is naturally attenuated until postnatal day (PN) 10 (Landers & Sullivan, 2014). However, the ontogeny of fear learning in phenotypically anxious organisms is mostly unknown. To address this issue, we used a selectively-bred anxiety-prone rat model, in which spontaneous anxiety-like behaviors emerge as early as at PN 11 (Maras et al., SFN Abstracts, 2014). Sprague-Dawley (SD) PN 4 rat pups selectively-bred for anxiety received 11 US electric shocks (0.4 mA, 1 s) to the tail, either paired with a CS peppermint odor (30 s) (Paired), or unpaired (Unpaired). Another group included pups that received 11 CS presentations (CS Only). Additional controls included wild-type SD pups matched for age and experimental conditions. At PN 11, all pups were re-exposed to 3 CSs and their behavior was videorecorded and scored for freezing. Analysis of variance (ANOVA) revealed no significant differences in freezing among the wild type animals ($p>0.05$), a finding consistent with previous studies using similar training parameters. However, the anxiety-prone Paired pups showed significantly higher levels of freezing as compared to the control groups (Unpaired and CS Only pups expressed comparable levels of freezing) (ANOVA: $F(3,46)=16.51$; $p<0.0001$; post hoc: $p<0.05$). This pattern of results demonstrates that fear conditioning in phenotypically anxious pups occurs very early in life, before they spontaneously express anxious behaviors at PN 11, and before the emergence of adult-like fear conditioning in wild-type rats at PN 10. In subsequent experiments we characterized the neuroendocrine mechanisms of infant fear learning in phenotypically anxious rats.

Disclosures: D. Chang: None. **J. Hider:** None. **P. Blandino:** None. **R.M. Sullivan:** None. **H. Akil:** None. **J. Debiec:** None.

Poster

694. Fear and Anxiety: Behavior

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Topic: F.03. Motivation and Emotion

Support: NIGMS 8P20GM103471-09

State of Nebraska LB692

Title: Differential gene expression after contextual fear conditioning in a novel fearful DxH recombinant inbred mouse strain

Authors: R. WICKRAMASEKARA[1], J. BOUMA[1], K. BEISEL[2], *D. M. YILMAZER-HANKE[2];
[2]Biomed. Sci. Dept., [1]Creighton Univ., Omaha, NE

Abstract: Emotions in response to anticipated threats are evolutionarily conserved to ensure the survival of species. However, pathological forms of fear and anxiety can lead to negative emotional and social consequences, causing high individual and socioeconomic burden. Current anxiolytic medications and exposure psychotherapy have variable outcomes in the individuals affected suggesting a role of genetic differences in biological mechanisms that drive fear and anxiety. In an attempt to understand potential genetic mechanisms of anxiety and fear, we have produced a congenic-like C3H-like recombinant inbred (C3HLRI) mouse strain with a high fear phenotype on a DBA/2J genetic background. This was achieved by backcrossing C3H/HeJ mice selected for a high fear sensitized acoustic startle response (FSS) onto DBA/2J mice with a low FSS with the goal of identifying genes related to high fear in the genetic background of low fear mice. In the present study, contextual fear conditioning was studied in fearful C3HLRI and control DBA/2J mice. Gene expression was analyzed in the hippocampus (HIP) six hours after fear training using microarrays. Mice from the contextual fear training group were presented footshocks and control animals exposed to the same context in the absence of shocks. Results obtained with Affymetrix Expression Console and Transcriptome Analysis Console indicated a limited number of differentially expressed genes. A subsequent two-way ANOVA differentiated between strain- and fear training-induced gene effects, which were further investigated with pathways analysis software. Among others, the genes identified included transcription factors, members of the cytoskeleton, synaptic proteins, genes regulating the cell cycle and cell death, ion channels, ion binding proteins, transporters, and enzymes. In summary, the results from bioinformatics analysis of our microarray expression data including analyses of potential molecular pathways involved suggest distinct mechanisms regulating strain effects and contextual fear learning in our congenic-like mouse strains. Further elaboration of these genes and molecular pathways will give us insights into genetic mechanisms of contextual fear and its extinction and may help to understand differential responses of patients to the treatment of anxiety disorders and related conditions.

Disclosures: **R. Wickramasekara:** None. **J. Bouma:** None. **K. Beisel:** None. **D.M. Yilmazer-Hanke:** None.

Poster

694. Fear and Anxiety: Behavior

Location: Hall A

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Program#/Poster#: 694.13/N3

Topic: F.03. Motivation and Emotion

Title: Galanin administration in the medial prefrontal cortex suppresses expression of conditioned fear memory and modulates plasticity during fear extinction

Authors: D. BHATTI, J. M. SMITH, *P. V. HOLMES;
Univ. Georgia, Athens, GA

Abstract: Posttraumatic Stress Disorder (PTSD) is characterized by a significant impairment in inhibiting learned fear. The goal of exposure therapy is to reduce conditioned fear responses by repeated presentation of a non-reinforced conditioned stimulus. Although exposure therapy is effective for many people with anxiety disorders, it is important to develop strategies to facilitate extinction of the fear response and enhance retention of this learning to improve clinical outcome. The neuropeptide galanin has anxiolytic and neurotrophic potential, but despite its known role in modulating anxiety-like behavior, galanin's potential for facilitating the extinction of aversive memories has not yet been explored. We investigated the effects of galanin administration in the mPFC of male Sprague-Dawley rats on facilitating extinction in a contextual fear-conditioning paradigm. Galanin (acute: .6 nmol in .5 ul/side; chronic: 3 nmol/day) or aCSF was either injected immediately before the first extinction trial or continuously infused (.25 ul/hr) into the bilateral mPFC for 3 weeks via an Alzet osmotic mini pump. For conditioning, rats were placed in an open field for 7 min and received three footshocks (.8 mA, .5 s) on minutes 4, 5, and 6, or received no footshock. 24 and 48 hours after conditioning, rats were placed back in the open field for 10 min. Freezing behavior, defined as the absence of all movements except those required for respiration or scanning, was scored for all trials. Rats were euthanized on day 4; rats that received chronic galanin were tested on the elevated plus maze (EPM) before euthanasia. The mPFC was dissected and PSD95 protein was measured by ELISA kit. Acute galanin administration reduced freezing during the first extinction trial ($p=.036$), but did not affect retention of extinction learning. This may be due to general inhibition of the prelimbic cortex, which is necessary for the expression of fear, or the anxiolytic effects of galanin, as chronic galanin administration, but not aCSF, increased the percent of time

on the open arm of the EPM (p=.0049). Furthermore, shock-naïve rats and rats that underwent fear conditioning and galanin administration expressed significantly lower PSD95 protein than those administered vehicle (p=.049). Our results suggest that galanin suppresses the expression of fear memory without affecting extinction learning and that galanin may modulate prefrontal cortical plasticity occurring during fear extinction.

Disclosures: D. Bhatti: None. **J.M. Smith:** None. **P.V. Holmes:** None.

Poster

694. Fear and Anxiety: Behavior

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Topic: F.03. Motivation and Emotion

Support: IN204314 from Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México (UNAM).

220173 from Consejo Nacional de Ciencia y Tecnología

Title: Selective ablation of the intercalated neurons of the amygdala increased the anxiety-like behavior in the Elevated Plus Maze

Authors: *E. PALOMARES[1], O. HERNANDEZ PEREZ[2], M. CRESPO RAMÍREZ[2], R. AGUILAR ROBLERO[2], K. FUXE[3], M. PÉREZ DE LA MORA[2];
[2]Cognitive Neurosciences, [1]Inst. de Fisiología Celular, Mexico City, Mexico; [3]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: The intercalated (ITC) islands of the amygdala are clusters of inhibitory neurons that surround the basolateral complex (BLA) and contain a dense population of dopamine D1 and μ-opioid receptors. Lateral ITC (lITC) islands provide feed-forward inhibition to the BLA, whereas medial ITC (mITC) islands form an inhibitory interface between the BLA and central nucleus (CeA), the main output region of the amygdala. Previous studies have shown that ITC neurons play a role in fear extinction. However the functional role of the ITC islands in the un-conditioned anxiety has not been studied. To elucidate the involvement of the ITC islands in the anxiety-like behavior in the Elevated Plus Maze, we bilaterally infused the toxin saporin conjugate with the agonist of the μ-opioid receptors, dermorphine, (SAP-DER; 0.75pmol/0.250μl/lado) in closed proximity to the mITC islands to specifically ablate the neurons of the ITC islands. Behaviorally, SAP-DER injections significantly increased the time

that the rats spent in the open arm of the maze as compared with their lesion control group. No effects on locomotion in the open-field test were found. These results suggest that ablate of the ITC neurons results in anxiogenic effects and support ITC neurons play an important role in mediate anxiolytic responses.

Disclosures: E. Palomares: None. **O. Hernandez Perez:** None. **M. Crespo Ramírez:** None. **R. Aguilar Roblero:** None. **K. Fuxe:** None. **M. Pérez de la Mora:** None.

Poster

694. Fear and Anxiety: Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 694.15/N5

Topic: F.03. Motivation and Emotion

Support: 1ZIAAT000023-02

Title: Persistent inflammatory pain reduces sexually motivated hedonic behavior in male rats

Authors: *M. H. PITCHER[1], F. TARUM[2], M. LEHMANN[3], M. BUSHNELL[2];
[2]Natl. Ctr. for Complementary and Integrative Hlth., [3]Natl. Inst. of Mental Hlth., [1]NIH, Bethesda, MD

Abstract: Anhedonia, or the loss of interest in previously rewarding or pleasurable activities such as sexual activity, is a diagnostic criterion for major depressive disorder. In male rodents, scent marking of urine from pro-estrous females is a hedonic behavior that is sensitive to stressors such as social defeat. Considering that depression is often comorbid with chronic pain in humans and depressive-like behavior may also be seen in rodent models of persistent pain, we tested whether or not urine scent marking behavior in rodents is altered in persistent inflammatory pain states. We compared urine scent marking behavior in rats (n=12/group) at baseline and one week after unilateral intra-articular injection of Complete Freund's Adjuvant (CFA) or sham injection. Male rats were allowed to scent mark in a large arena with an absorbent paper floor containing a spot of urine harvested from pro-estrous female rats. Scent marking behavior was assessed by measuring the area of scent marking as well as time spent in a circular zone around the female urine spot as well as in the remaining arena. At baseline (prior to CFA or sham injection), males in both the CFA and sham groups exhibited a robust preference for marking the female marked zone, with 51% and 47% more marking in this zone than in the remaining arena, respectively. While scent-marking behavior was unchanged one week after sham injection, intra-articular CFA significantly reduced marking preference by approximately

35% (33.16±6.02%, *p=0.030*). However, the amount of time spent in the female marked zone increased by 21% following CFA (*p=0.036*). These findings demonstrate that inflammatory pain alters hedonic behavior in rats. In the context of Fields' Motivation-Decision model of pain and pleasure, while persistent pain may reduce the communicative component of hedonic behavior (i.e. reduced urine scent marking) to avoid potential conspecific aggression, there is also a concomitant increase in seeking out hedonic signals (i.e. increased time in the female-marked zone). As such, urine scent marking behavior may represent an ethologically relevant assay supporting translational research of persistent pain-induced depression.

Disclosures: M.H. Pitcher: None. **F. Tarum:** None. **M. Lehmann:** None. **M. Bushnell:** None.

Poster

694. Fear and Anxiety: Behavior

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

Support: NIH Grant 1RO1MH080194

Title: Maternal TNF during gestation programs the balance between risk taking and avoidance

Authors: *B. LIU[1], B. ZUPAN[2], M. TOTH[1];
[1]pharmacology, Weill Cornell Med. Col., New York, NY; [2]Vassar Col., Poughkeepsie, NY

Abstract: TNF is a cytokine with roles not only in coordinating local and systemic immune responses but also regulatingneuronal functions.Our previous research showed that maternal TNFduring early postnatal life programsdorsal hippocampal proliferation and the level of adult spatial memory. Here we report a new neurobiological function forTNF; specifically, the requirement for maternalTNFduring pregnancy to establish "normal" risk-avoidant behaviorin the offspring. We found that the adult offspring exposed to TNF deficient maternal environment during their development exhibit less avoidance and more risk taking behavior in a novel, anxiety-inducing environment. Cross-fostering and conditional knockout experiments indicated that TNF deficit in the maternal brain, rather than in the hematopoietic system, and during the prenatal period was responsible for theoffspring phenotype.Our data show a role for maternal brain TNF in programming offspring emotional response to risk.

Disclosures: B. Liu: None. **B. Zupan:** None. **M. Toth:** None.

Poster

694. Fear and Anxiety: Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 694.17/N7

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

Support: State of Florida

Title: Exposure to social defeat stress increases morphine consumption and depression-like behavior mediated by the endogenous kappa opioid system

Authors: *K. A. HYMEL[1,2], J. M. MEDINA[1,2], S. O. EANS[1,2], M. L. GANNO[2], H. M. STACY[2], J. P. MCLAUGHLIN[1,2];
[1]Univ. of Florida, Gainesville, FL; [2]Torrey Pines Inst. for Mol. Studies, Port St. Lucie, FL

Abstract: Post-traumatic stress disorder (PTSD) often presents with comorbid substance abuse. Exposure to social defeat stress (SDS), an animal model of PTSD, is known to potentiate cocaine conditioned place preference through a kappa-opioid receptor (KOR) dependent mechanism. The current study examined the effects of SDS on morphine consumption in a two bottle choice paradigm using C57BL/6J and KOR KO mice. After four days' habituation to quinine (0.25 mg/mL) in each bottle, individually housed mice were allowed free access for four days to both quinine and morphine sulfate, demonstrating consistent consumption of the opioid each day at a dose of 1 μg/mL morphine. Mice demonstrating daily stable preference for the morphine bottle then underwent a 4-day SDS procedure as the intruder in the home cage of a CD-1 male aggressor for 20 min, twice a day (Day 9-12). Control mice instead explored a novel environment. Bottles were weighed daily to quantify consumption, and time spent in socially defeated postures was examined. Depression-like behavior was evaluated the day following the last SDS exposure (Day 13) in a 15 min forced swim test. Consumption continued to be measured 4 days after SDS exposure had concluded (Days 13-16). During exposure to SDS, C57BL/6J mice showed a significant 1.6-fold increase in morphine consumption relative to their non-stressed littermates, but this effect was considerably reduced in KOR KO mice. In addition, SDS-exposed C57BL/6J, but not KOR KO, mice demonstrated significantly greater time spent immobile in the forced swim test compared to their non-stressed counterparts. Notably, following the conclusion of the stress procedure, morphine consumption of SDS-exposed C57BL/6J mice returned over four days to levels equivalent to that of non-stressed controls. In contrast, no change in consumption was observed between the stress-exposed and unstressed KOR KO mice. These preliminary findings suggest that SDS-induced increases in morphine consumption are mediated via the activity of the kappa opioid receptor, further suggesting the

endogenous kappa opioid system as a therapeutic target to treat PTSD and other stress-related disorders with comorbid substance abuse.

Disclosures: **K.A. Hymel:** None. **J.M. Medina:** None. **S.O. Eans:** None. **M.L. Ganno:** None. **H.M. Stacy:** None. **J.P. McLaughlin:** None.

Poster

694. Fear and Anxiety: Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 694.18/N8

Topic: F.03. Motivation and Emotion

Support: UFSCar

CNPQ

FAPESP

Title: Empathy for Pain: Mice undergoing neuropathic pain induce specific effects on pain response in cagemates subjected to various nociceptive tests

Authors: *D. B. SOUZA[1], R. L. NUNES-DE-SOUZA[2], A. L. M. CANTO-DE-SOUZA[1]; [1]Univ. Federal De São Carlos, São Carlos, Brazil; [2]UNESP, Araraquara, Brazil

Abstract: AIM: The ability of an individual to identify emotional signals in conspecifics, a phenomenon called empathy, is directly related to the survival of the species. Although usual in human being, empathy has also been observed in animals. For instance, it has been shown that pain-related behaviors are bidirectionally influenced among animals that are suffering pain. In other words, the sight of a rodent suffering pain enhances the nociceptive response displayed by the observer cagemate. Recently, our research group has shown that mice living with a conspecific suffering with chronic pain [e.g., pain induced by sciatic nerve constriction (SNC)] display increased nociceptive responses assessed with the writhing test. Here we investigated whether this type of empathy is also observed when formalin test and hot plate test are employed to assess pain response in the observer mouse living with a cagemate suffering of chronic pain (SNC). METHODS AND RESULTS: Male Swiss mice (n=6-8 per group) were housed in pairs for 28 consecutive days. On day 14th, pairs of mice were grouped as follow: Cagemate (CNC), in which one animal from each pair was subjected to SNC surgery; Cagemate sham (CS), in which one animal from each pair was subjected to SNC sham surgery. After that, each pair was returned to its homecage to live together for further 14 days. On testing day (day 28th): the

observer cagemate (i.e. of each pair was subjected to one of the following nociceptive tests: Writhing test, Formalin test or Hot plate test. Student t-test revealed that the CNC groups displayed higher number of abdominal writhes ($t(9) = 8.64$, $P<0.05$) and lower time spent licking the formalin injected paw ($t(13) = 1.04$, $P<0.05$) than animals from CS group. No significant differences were observed between CNC and CS groups on the latency to lick the paw in mice exposed to the hot plate test ($t(12) = 5.81$, $P>0.05$). CONCLUSION: These results demonstrated that living with animals subjected to SNC induces hyperalgesia and hypoalgesia in the observer cagemate, as assessed in the writhing test and formalin test, respectively. Taken together, the present results suggest using of the writhing test to study the neurobiology of empathy in mice.

Disclosures: D.B. Souza: None. **R.L. Nunes-de-Souza:** None. **A.L.M. Canto-de-Souza:** None.

Poster

694. Fear and Anxiety: Behavior

Location: Hall A

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Program#/Poster#: 694.19/N9

Topic: F.03. Motivation and Emotion

Support: UFSCar

CNPQ (307238/2012-1)

CAPES

FAPESP (2009/17938-6, 2013/22284-0)

Title: Mice undergoing neuropathic pain induce anxiogenic-like effects and hyperalgesia in cagemates

Authors: *A. CANTO-DE-SOUZA[1,2,3], A. C. NUNCIATO[1,3], B. C. PEREIRA[1,2], C. R. ZANIBONI[1], G. FACHINI[1,3], D. BAPTISTA-DE-SOUZA[1,3];
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Abstract: AIM: As previously described, conspecific rodents are able to respond pain-related responses in cagemates subjected to nociceptive tests. Therefore, cohabitation with a conspecific animal with chronic pain is potentially capable of promoting a stressful situation, which can

trigger behavioral changes such as anxiety and depression and alter nociceptive responses. In the present study we investigated the effect of cohabitation with a mouse undergoing sciatic nerve constriction (neuropathic pain model). The cagemates were evaluated on nociception (writhing test), anxiety (elevated plus-maze and open field), depression (forced swim, tail suspension and sucrose preference test) and the corticosterone levels. METHODS AND RESULTS: Male Swiss mice (n=6-12 per group) were housed in pairs for 28 consecutive days. On day 14th, pairs of mice were grouped as follow: Cagemate (CNC), in which one animal from each pair was subjected to sciatic nerve constriction (SNC) surgery; Cagemate sham (CS), in which one animal from each pair was subjected to SNC sham surgery. After that, each pair was returned to its homecage to live together for further 14 days. On testing day (day 28th): the observer cagemate (i.e. of each pair) was subjected to one of the following tests: Experiment 1, writhing test; Experiment 2, elevated plus-maze (EPM) and open field tests; Experiment 3, forced swim, tail suspension, sucrose preference test and Experiment 4, corticosterone levels. Our results demonstrated that the social interaction of conspecific undergoing SNC induced hypernociception ($F(2.25)=12.00$, $P<0.05$), increased anxiety-related responses [EPM: percent open arm entries - %OA ($F(2.32)= 11.67$, $P<0.05$), percent of time spent in open arms - %tOA ($F(2.32)= 13.24$, $P<0.05$); Open field: percent of time spent in central area ($F(2.21)= 13.10$, $P<0.05$-Experiment 2]; while no changes in depression tests [Forced swim test: total immobility ($F(2.23)=3.59$, $P<0.05$) or latency ($F(2.23)=6.50$, $P<0.05$); Tail suspension test: immobility ($F(2.39)=3.59$, $P>0.81$) or latency ($F(2.39)=6.50$, $P>0.55$); Sucrose preference test: sucrose preference ($F(2.20)=0.78$, $P>0.05$)- Experiment 3] and the corticosterone levels [($F(2.14)=0.31$, $P>0.05$)- Experiment 4] were found. CONCLUSIONS: These results demonstrated that cohabitation with suffering conspecifics (SNC) induces hypernociception and anxiogenic-like effects in mice cagemates. Together, the present results suggest using of the writhing test and elevated plus-maze to study the neurobiology of empathy in mice.

Disclosures: **A. Canto-de-Souza:** None. **A.C. Nunciato:** None. **B.C. Pereira:** None. **C.R. Zaniboni:** None. **G. Fachini:** None. **D. Baptista-de-Souza:** None.

Poster

694. Fear and Anxiety: Behavior

Location: Hall A

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Program#/Poster#: 694.20/N10

Topic: F.03. Motivation and Emotion

Support: CAPES-PNPD 2639/2011

CNPq 307238/2012-1

Title: Blockade of medial prefrontal cortex mineralocorticoid receptors impairs defensive reaction in mice exposed to the olfactory fear conditioning

Authors: *R. R. SOUZA[1], V. F. CAMPOS[2], A. CANTO-DE-SOUZA[2];
[1]Univ. Federal De Sao Carlos, Sao Carlos, Brazil; [2]Dept. of Psychology, Univ. Federal de Sao Carlos, São Carlos, Brazil

Abstract: AIM: Central mineralocorticoid receptors (MR) are nuclear and membrane receptors of primary importance for stress-related response and adaptation towards environmental threats. The MR are highly expressed in the medial prefrontal cortex (mPFC), a key area for memory and coping with stressors. In the present study we investigated de effects of systemic or intra-mPFC MR antagonists on the behavior of mice exposed to an olfactory fear conditioning task (OFC). METHODS AND RESULTS: Male Swiss mice (n=8-10/group) were exposed the OFC protocol using a two-side conditioning chamber (A) and an odor-exposure chamber (B). In the Experiment 1, defensive behaviors were measured in non-shocked, non-paired, or odor-paired mice exposed to the conditioned odor (chamber B) and to the conditioned context (chamber A) at different days (24h interval). In the Experiment 2, different groups of odor-paired mice received systemic injections of vehicle or the MR antagonist spironolactone (SPI, 10-30 mg/kg, i.p.) 30 minutes prior the exposure to the conditioned odor or to the conditioned context. In the Experiment 3, odor-paired mice received intra-mPFC infusions of vehicle or the MR antagonist RU20318 (5-10 ng/0.1µl/side) 5 minutes before the exposure to the conditioned odor. For Experiment 1, we found that shock-paired mice exposed to the conditioned odor (B) presented an increased time in the burrow and tunnel zones, and a reduced time in the open surface, approach zone and climbing the grid wall in comparison to non-shocked, or to non-paired mice. Shock-paired mice also showed significantly higher time in SAP and burying, and a reduced frequency of rearings and transitions. Upon the exposure to the conditioned context (A), all mice that received footshock presented a reduction in rearings and transitions, as well as an increase in freezing and a reduced time in the conditioned compartment. For Experiment 2, systemic injection of SPI (10 or 30 mg/kg) did not change spatiotemporal measurements, but significantly reduced the time in SAP and burying, as well as increased the number of rearings. However, injections of SPI did not change behavioral measurements in the conditioned context. For Experiment 3, intra-mPFC infusion of RU28318 (5 or 10 ng) prior the exposure to the conditioned odor, significantly reduced the time in the burrow, as well as the time in SAP and burying. CONCLUSIONS: Altogether, the present study supports the usefulness of the olfactory fear conditioning and suggests that mPFC mineralocorticoid receptors are critically involved in the coping strategy and orchestration of defense reaction towards olfactory fear cues.

Disclosures: **R.R. Souza:** None. **V.F. Campos:** None. **A. Canto-de-Souza:** None.

Poster

694. Fear and Anxiety: Behavior

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Program#/Poster#: 694.21/N11

Topic: F.03. Motivation and Emotion

Support: FAPESP 2013/01283-6

CNPq 305597/2012-4

CNPq 473102/2009-9

CAPES 2053/2013

Title: Chemical inhibition of the left medial prefrontal cortex facilitates anxiety-related behavior in mice subjected to social defeat (but not to restraint) stress

Authors: *R. L. NUNES-DE-SOUZA[1], N. S. COSTA[2], M. A. VICENTE[2];
[2]Pharmacol., [1]Univ. Estadual Paulista, UNESP, Araraquara, Brazil

Abstract: Recent results from our lab have shown that chemical inhibition (e.g., with cobalt chloride, CoCl2) of the left medial prefrontal cortex (LmPFC) produces anxiogenic-like effect in mice exposed to the elevated plus maze (EPM), a widely used animal model of anxiety. Interestingly, similar effect was obtained with injection of NOC-9, a nitric oxide (NO) donor, injected into the right mPFC (RmPFC), suggesting a functional lateralization of the mPFC in the modulation of anxiety-related behavior (Nunes-de-Souza et al., in Neuroscience 2014). It has been suggested that the LmPFC could play an inhibitory role on the RmPFC, reducing the deleterious effects of stressors on emotional states. Here, we investigated the effects of intra-LmPFC injection of CoCl2 on anxiety-related behavior of mice previously exposed to two different stressors: restraint stress or social defeat stress. Male Swiss mice (n=10-20) received intra-LmPFC injection of saline or CoCl2 (0.1mM/0.2µL) 10 minutes before being subjected to (i) 30-min restraint stress or (ii) social defeat stress. 24h later, each animal was exposed to the EPM for a period of 5 minutes, when the conventional anxiety measures (percentage of open-arm entries and percentage of open-arm time: %OE and %OT) and locomotor activity (frequency of closed-arm entries: CE) were recorded. Control groups received either saline or CoCl2 and were not exposed to stress situation. Results [two-way ANOVA followed by Duncan test; Factor 1: Condition (no stress or stress); Factor 2: Treatment (saline or CoCl2)] revealed an important effect for condition x treatment interaction, showing that intra-LmPFC injection of CoCl2 produced anxiogenic-like effects only in socially defeated mice, reducing the open arm exploration [%OE ($F(2,61)=3.88$; $p<0.05$); %OT ($F(2,61)=4.23$; $p<0.05$)]. Importantly, intra-LmPFC injections of CoCl2 did not change locomotor activity [CE ($F(1,61)=1.89$; $p>0.05$)].

These results suggest that a single exposure to the restraint or social defeat stress does not enhance anxiety in mice exposed to the EPM 24 hours later. However, inhibition of the LmPFC induces anxiogenic-like effects in socially defeated (but not restrained) mice. Together, these results suggest that the LmPFC plays a role in the resilience of mice to the anxiogenic effects provoked by social defeat.

Disclosures: R.L. Nunes-de-Souza: None. **N.S. Costa:** None. **M.A. Vicente:** None.

Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant U01 AA020912

NIH grant U01 AA016654

Title: Repeated binge drinking increases perineuronal nets in the insular cortex

Authors: *H. CHEN, D. HE, A. W. LASEK;
Psychiatry, UIC, Chicago, IL

Abstract: Perineuronal nets (PNs) are specialized extracellular matrix structures that enclose subpopulations of neurons in the brain. Intragastric administration of alcohol in adolescent mice induces long-lasting increases of PN components in the orbital cortex in adulthood. However, it has not been determined whether binge alcohol exposure in young adults alters PNs. Here we examined PNs and their core components in the insula and primary motor cortex after repeated binge-like ethanol consumption in adult male mice. We performed the 4 day drinking in the dark (DID) procedure for 1 or 6 weeks to model binge alcohol consumption. The impact of ethanol drinking on PNs was examined by fluorescent staining of brain sections using a marker for PNs, Wisteria floribunda agglutinin (WFA). In another set of experiments, cortex was dissected and Western blots and quantitative real-time PCR (qPCR) were performed to evaluate the expression of PN proteins aggrecan, brevican, and phosphacan. We found that 6 weeks of ethanol DID significantly increased PN intensity in the insula, as measured by WFA binding. Aggrecan, brevican and phosphacan protein expression, and aggrecan mRNA expression, were also elevated in the insula after 6 weeks of ethanol drinking. In contrast, expression of PN components did not change after 1 week of DID. The increase in PNs appears to be specific to

the insula, since alterations were not observed in the primary motor cortex. Our results provide the first evidence that insular PNs increase after long-term binge drinking. The insula mediates compulsive alcohol use. Since PNs influence neuronal firing and plasticity, increased PNs in the insula after multiple binge cycles may contribute to restricted neuronal plasticity and lead to the development of compulsive alcohol use.

Disclosures: H. Chen: None. **D. He:** None. **A.W. Lasek:** None.

Poster

695. Alcohol: Neural Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 695.02/N13

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH AA023291

Title: Alterations in ethanol taste-elicited neuronal activity in the insular cortex following chronic ethanol exposure

Authors: E. DRIVER, B. FERETIC, J. PENPRASE, J. GODFREY, C. QUINTANILLA, *S. M. BRASSER;
San Diego State Univ., San Diego, CA

Abstract: Chronic exposure to alcohol produces neuroadaptations in the central nervous system that contribute both to the maintenance of ongoing drug use and persistent vulnerability to relapse. Part of this neuroplasticity may involve sensitization to stimulus cues that are repeatedly and reliably associated with drug administration. Due to its oral route of administration, the chemosensory cues accompanying alcohol consumption are among the most intimate and consistent stimuli immediately predictive of the drug's postabsorptive effects. In alcoholics and high risk drinkers, alcohol chemosensory stimuli elicit urges to drink as well as activation of mesocorticolimbic structures implicated in drug seeking and motivation. In animal models of relapse, re-exposure to ethanol taste cues following extinction of ethanol self-administration induces strong reinstatement of ethanol seeking. Recent findings have implicated the insular cortex as a significant brain region of interest involved in the processing of drug-associated stimuli. Human imaging studies have revealed insular activation during the experience of drug-related craving and exposure to drug-associated cues, and inactivation of the insula in rodents disrupts conditioned preference for environments previously paired with nicotine or amphetamine. The present study investigated alterations in the neural processing of ethanol

orosensory signals within the insular cortex following chronic experience with the drug, as measured via neuronal Fos response elicited by ethanol chemosensory stimuli in animals with and without a history of exposure. Outbred Wistar rats were chronically exposed to either a 20% ethanol or 0.5% saccharin intermittent access drinking paradigm or were given access only to water. Following the chronic drinking phase, naive and experienced rats from each stimulus condition were allowed to consume a fixed volume of their respective exposure stimulus (20% ethanol or 0.5% saccharin) and neuronal Fos response within the gustatory insula was measured 90 min post-exposure. Oral administration of 20% ethanol produced robust activation of the gustatory insula in naive rats, which was significantly reduced in alcohol-experienced animals. A trend for a similar reduction in tastant-elicited Fos expression was observed in saccharin-exposed subjects. These data suggest that gustatory regions of the insula may process information relevant to the novelty/familiarity of ethanol taste similar to other appetitive tastants.

Disclosures: E. Driver: None. **B. Feretic:** None. **J. Penprase:** None. **J. Godfrey:** None. **C. Quintanilla:** None. **S.M. Brasser:** None.

Poster

695. Alcohol: Neural Mechanisms

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Program#/Poster#: 695.03/N14

Topic: C.17. Drugs of Abuse and Addiction

Support: Supported by AA019682

Title: Chemogenetic inactivation of the insular cortex increases interoceptive sensitivity to alcohol

Authors: *A. A. JARAMILLO, P. A. RANDALL, Z. A. MCELLIGOTT, J. BESHEER; Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Previous work has demonstrated a role for the nucleus accumbens core (AcbC) in regulating the interoceptive/subjective effects of alcohol. In order to investigate potential novel circuits involved in modulating sensitivity to alcohol, male Long-Evans rats (n=12) received a microinjection of the retrograde tracer Fluorogold (FG) directed at the AcbC. Projections to the AcbC were visualized through immunohistochemistry. Dense FG immunoreactivity (IR) was found in the insular cortex (IC). Next, to begin to examine a possible role for IC projections to AcbC (IC→AcbC) in modulating sensitivity to alcohol, we examined neuronal response to alcohol (1 g/kg, IG). Examination of co-localized FG and c-Fos IR revealed an alcohol-induced

increase in c-Fos IR within FG positive cells, demonstrating an IC→AcbC specific response to alcohol. Next, to validate the use of a chemogenetic approach by which to inactivate the IC, rats were microinjected with inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs; hM4D(Gi)) in the IC. DREADD expression and functional validation were confirmed with IR and electrophysiological recordings. To determine the functional role of the IC in modulating sensitivity to alcohol, male Long-Evans rats (n=6-9) were microinjected with the Gi DREADDs in the IC and trained to discriminate alcohol (1 g/kg, IG) vs. water using standard drug discrimination techniques. Rats received pretreatment with 1 mg/kg clozapine-N-oxide (CNO; IP) to activate the Gi DREADDs, 45 min prior to a cumulative alcohol curve (0.1, 0.3, 1.0, and 1.7 g/kg). Chemogenetic activation of the Gi DREADDs following CNO pretreatment resulted in increased sensitivity to low alcohol doses (0.1 - 0.3 g/kg), demonstrating a role of the IC in regulating the interoceptive effects of alcohol. Further these data demonstrate the feasibility of investigating a striatal-insula circuit in modulating sensitivity to alcohol.

Disclosures: **A.A. Jaramillo:** None. **P.A. Randall:** None. **Z.A. McElligott:** None. **J. Besheer:** None.

Poster

695. Alcohol: Neural Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 695.04/N15

Topic: C.17. Drugs of Abuse and Addiction

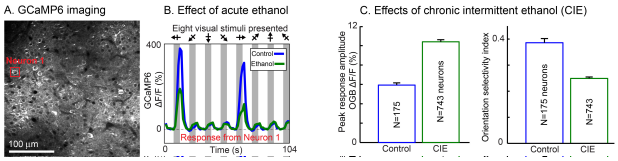
Support: NIH R21 AA022168

Title: *In vivo* two-photon imaging reveals modulation of synaptic and spiking activity in visual cortex following acute and chronic exposure to alcohol

Authors: P. O'HERRON, M. LEVY, M. F. LOPEZ, J. J. WOODWARD, *P. KARA; Neurosci., MUSC, Charleston, SC

Abstract: The medial and orbital regions of the prefrontal cortex along with their direct projection and recipient zones are a major focus of research on the effects of alcohol in the brain. However, although glutamate receptor-dependent plasticity and reward-related activity are found in the visual cortex, this and other sensory neocortical regions are largely ignored in animal models of alcohol dependence. Here we test the hypothesis that responses of neurons in the visual cortex to sensory stimulation are altered after acute and chronic bouts of alcohol exposure. We used two-photon imaging in the mouse primary visual cortex to measure spiking and

synaptic responses by labeling neurons with synthetic and genetically encoded fluorescent sensors (Fig. panel A). Fluorescent calcium indicators OGB-1 AM and gCaMP6s were used to measure spiking activity while a glutamate sensor iGluSnFr was used to track synaptic activity. We first applied ethanol locally to the visual cortex in order to avoid potential effects on retinal and thalamic neurons that could occur via systemic injections. Acute alcohol produced an overall reduction of visually evoked spiking activity (Fig. panel B) and synaptic responses (not shown). We then tested whether this depression of visual responses persisted in mice exposed to four weekly cycles of chronic intermittent ethanol (CIE) vapor exposure in an inhalation chamber. Imaging was performed 72 hr after withdrawal from the last CIE treatment. Surprisingly, we found that CIE treatment increased the amplitude of sensory-evoked cortical responses. This increased response amplitude across a range of stimulus orientations resulted in a marked decrease in orientation selectivity (Fig. panel C). Thus, with CIE treatment, cortical neurons appear to become more promiscuous to sensory stimuli. In summary, our data suggest that sensory cortex undergoes lasting changes in neural circuitry related to alcohol dependence and that these persistent effects appear to be opposite to those produced by acute ethanol.



Disclosures: **P. O'Herron:** None. **M. Levy:** None. **M.F. Lopez:** None. **J.J. Woodward:** None. **P. Kara:** None.

Poster

695. Alcohol: Neural Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 695.05/N16

Topic: C.17. Drugs of Abuse and Addiction

Title: Orbitofrontal cortex neurons are activated during alcohol and sucrose self-administration

Authors: ***J. HERNANDEZ**, D. MOORMAN;
Univ. of Massachusetts - Amherst, Amherst, MA

Abstract: The orbitofrontal cortex (OFC) is involved in reward valuation and preference, and OFC disruption has been associated with addiction to drugs of abuse. Recent work has shown that alcohol exposure has pronounced effects on OFC neuron excitability and plasticity.

However, it is unknown how OFC neurons signal alcohol reward and motivation in behaving animals. Characterizing the contributions of OFC networks to alcohol reward has the potential to provide a mechanistic understanding of critical neural systems that drive motivation for alcohol in both addicted and non-addicted states. In a preliminary set of results, we found that a subset of OFC neurons are strongly modulated during a basic cue-reward test of ethanol seeking. However, we and others have also shown that OFC neurons in the rat are robustly activated during sucrose self-administration. It is unclear whether OFC activity changes in each task encode reward seeking in general, or whether neurons exhibit selectivity for alcohol or sucrose rewards. Thus in these studies we recorded OFC activity in a task that combined alcohol and sucrose seeking. Long-Evans rats received intermittent access to 20% ethanol in their home-cage for 4 weeks before being trained on a cue-driven combined alcohol/ sucrose-seeking task. Nosepoke-initiated trials triggered one of two auditory cues. Tone 1 (1 kHz) predicted delivery of 20% ethanol. Tone 2 (5 kHz) predicted delivery of 15% sucrose solution to the same location. Reward was delivered if the animal left the nosepoke in less than 500 ms of the cue and initiated licking the tube within 500 ms of leaving the nosepoke. Upon stable performance, we implanted unilateral 32 microwire arrays in medial and lateral OFC to record activity of OFC neurons during the sucrose/alcohol-seeking task. Both sucrose- and alcohol-predicting tones resulted in activation of OFC neurons. Responses were stronger for sucrose than for alcohol, likely reflecting the well-established OFC encoding of relative reward value (sucrose being preferable to alcohol). We are currently recording from animals with varying degrees of alcohol preference to assess the impact of preference on OFC alcohol vs. sucrose encoding. We are also comparing OFC activity in mixed sucrose/alcohol self-administration tasks (as above) to exclusively sucrose or alcohol tasks to investigate the influence of reward availability on relative encoding of sucrose vs. ethanol value.

Disclosures: **J. Hernandez:** None. **D. Moorman:** None.

Poster

695. Alcohol: Neural Mechanisms

Location: Hall A

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Program#/Poster#: 695.06/N17

Topic: C.17. Drugs of Abuse and Addiction

Support: NHMRC Grant 1061979

Title: Investigating changes in medial prefrontal cortex and basolateral amygdala neuronal morphology following long-term ethanol consumption

Authors: *P. M. KLENOWSKI[1], M. SHARIFF[1], A. BELMER[1], M. FOGARTY[2], M. BELLINGHAM[2], S. BARTLETT[1];
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Abstract: Repeated binge alcohol drinking followed by abstinence are key factors contributing to the development of dependence. Long-term alcohol consumption changes reward pathways in the brain and also alters neuronal circuits that control behavioral responses during abstinence. This causes sensitization to negative emotional states of withdrawal such as anxiety and stress, which contributes to alcohol craving and relapse. The activity of the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) altered by long-term alcohol use and are thought to contribute to the enhancement of alcohol withdrawal symptoms, however limited data is available regarding modifications in morphology and synaptic connectivity that underpin changes in this neural circuit. Therefore, the aim of this study was to assess the effect of long-term ethanol consumption on the morphological characteristics of mPFC and BLA principal cells. We have filled several mPFC and BLA principal neurons with neurobiotin from rats given intermittent access to 20% ethanol for 10 weeks using a two-bottle choice paradigm and age-matched water controls. We have also recorded spontaneous synaptic currents and performed post-immunolabeling with antibodies against key pre- and post-synaptic components of GABAergic and glutamatergic synapses to determine changes in the activity and localization of inhibitory and excitatory putative synapses of mPFC and BLA principal neurons following repeated cycles of binge ethanol consumption. By determining changes in morphology, spontaneous synaptic activity and synaptic density of neurobiotin-filled mPFC and BLA principal neurons following long-term ethanol consumption, we will be able to uncover how changes in the connectivity of this neural circuit enhance withdrawal symptoms and contribute to the development of alcohol dependence.

Disclosures: P.M. Klenowski: None. **M. Shariff:** None. **A. Belmer:** None. **M. Fogarty:** None. **M. Bellingham:** None. **S. Bartlett:** None.

Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA Grant AA47159

NIAAA Grant AA07573

Title: mPFC modulates interoceptive sensitivity to a nicotine + alcohol compound cue: functional validation using designer receptors exclusively activated by designer drugs

Authors: *P. A. RANDALL[1], A. A. JARAMILLO[1,2], Z. MCELLIGOTT[1], J. BESHEER[1,2,3]; [1]Ctr. for Alcohol Studies, [2]Curriculum In Neurobio., [3]Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Nicotine and alcohol are two of the most common drugs regularly used in combination. As a result, the interoceptive effects of each drug are frequently experienced together and over time, develop into a unique interoceptive cue associated with other reinforcing events. Considering the substantial health risks of combined nicotine and alcohol use, increasing our understanding of functional brain regional involvement in modulating sensitivity to these interoceptive effects is important in developing better treatments. The medial prefrontal cortex (mPFC; prelimbic region) is known to play a role in different aspects of associative learning and drug taking behavior. As such, the current experiments sought to explore the role of the mPFC in modulating sensitivity to a nicotine+alcohol (N+A) compound interoceptive cue. To do so, these studies utilize designer receptors exclusively activated by designer drugs (DREADDs) to silence mPFC in rats trained to discriminate a N+A compound cue from water. The first set of experiments assessed the functional viability of using Cre-dependent AAV-hSyn-DIO-hM4D(Gi) DREADDs in mPFC using both electrophysiological and immunohistochemical methods (n=8). The second set of experiments assessed the effects of silencing mPFC on sensitivity to the N+A compound cue (n=20). Long-Evans rats received bilateral infusions of Cre-dependent AAV-hSyn-DIO-hM4D(Gi) into mPFC. Following recovery, rats were trained to discriminate a nicotine (0.4 mg/kg) + alcohol (1 g/kg) drug state vs. vehicle. 6 weeks post-DREADD infusion robust DREADD expression in the mPFC was observed as measured by IHC. Additionally, clozapine-N-oxide (CNO, 10 μM) significantly decreased measures of cellular activity as assessed by electrophysiology. In the behavioral studies, activation of the inhibitory DREADDs by CNO (1 mg/kg, IP) significantly decreased N+A related goal-tracking behavior at a dose that did not affect locomotor activity. Together these studies demonstrate the functional involvement of mPFC in modulating interoceptive sensitivity to the N+A compound cue.
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Poster

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Alcohol addiction impairs human hippocampal neurogenesis: Effects on proliferation, neuronal stem cells and immature neurons

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Abstract: Alcohol abuse is associated with neurodegeneration in the hippocampus, a region associated with learning, memory and mood regulation in humans. We hypothesized that alcohol may have detrimental effect on the neurogenic pool of stem cells and/or immature neurons in the dentate gyrus (DG) of the human hippocampus. Therefore, we investigated whether alcohol abuse affects the number of neuronal stem/progenitor cells, immature/migrating neurons and proliferating cells in postmortem human hippocampal samples isolated from deceased donors subjected to a forensic autopsy. Classification of subjects was based on amount of alcohol consumed the last 4 weeks before their demise, according to information from relatives, the forensic pathology investigations, police reports and medical records. Hippocampal sections from controls and alcoholics were immunostained for Sox2, a neuronal stem/progenitor cell marker, doublecortin, a marker for immature/migrating neurons, and Ki67, a marker of cell proliferation. Positively stained cells were counted in alcoholics and compared with age-matched controls. Counting was performed in whole DG, including the molecular layer (ML), the granular cell layer (GCL) and the subgranular zone (SGZ). We also counted cells separately in the SGZ. The number of cells immunoreactive for doublecortin, Sox2 and Ki67 was significantly reduced in the whole DG and in the SGZ in alcoholics as compared to controls. No correlation between the cell numbers expressing any of the three markers and subject age was observed. In summary, our data indicate that alcohol impairs neurogenesis in the human hippocampus, and suggest that pharmacological agents that act on the hippocampal stem cell pool may be particularly interesting to study in the search for drugs to treat alcohol addiction.

Disclosures: **G. Dhanabalan:** None. **T. Wardi Le Maitre:** None. **K. Alkass:** None. **H. Druid:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA T32AA00745

NIAAA AA020098

NIAAA AA06420

Title: Prolonged abstinence from chronic ethanol exposure impairs hippocampal cognition and plasticity within the hippocampus

Authors: *M. C. STAPLES, M. J. FANNON, C. D. MANDYAM;
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Abstract: Alcohol dependence is a condition including repeated cycles of excessive alcohol consumption and periods of abstinence. Impairments in hippocampal (HPC) cognition during alcohol exposure are well characterized, but the long-term effects following cessation of chronic ethanol exposure on HPC-sensitive cognitive function and corresponding measures of structural plasticity and plasticity associated proteins are unknown. A trace fear conditioning (TFC) protocol was used to assess hippocampal cognition during abstinence along with Golgi staining for granule cell structural analysis and western blotting for dentate gyrus (DG) specific glutamatergic receptor expression. Following 7 weeks of chronic intermittent ethanol (EtOH) vapor exposure (CIE, daily 14 hours EtOH and 10 hours air exposure) and 72 hours of withdrawal from EtOH exposure, animals were trained in TFC and tested 24h, 10d, or 21d later. The cortex of each animal was collected and either incubated in Golgi stain or micropunched in the dorsal and ventral DG for western blot analysis. CIE had no impact on acquisition of the task (measured as freezing during the trace periods following the tone presentation on the training day 72 after CIE cessation), or on the contextual recall at any of the three time points. Recall of the trace memory, assessed as freezing during the 1st trace period on the testing day, was significantly impaired by CIE at all three time points of testing demonstrating persistence of impairments in HPC cognition and a lack of recovery during prolonged abstinence. 3D Sholl analysis revealed that the structure of granule cells in the dorsal and ventral hippocampus of CIE animals were significantly altered compared to air controls during abstinence with most effects noted during protracted abstinence. Ongoing studies include biochemical analysis of glutamatergic receptor expression which may be underlying the structural plasticity differences and cognitive deficits observed in CIE animals. It can be concluded that as abstinence from EtOH continues, HPC cognitive performance remains impaired, and this impairment appears to be related to structural abnormalities observed across the time course of abstinence. Further investigations into the neurochemical underpinnings of this perturbed behavior should be conducted to better understand more precisely the deleterious impact of ethanol dependence.

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Poster

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Title: Chronic ethanol self-administration in female macaques disrupts presynaptic dopamine neurotransmission

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Abstract: Hypofunction of striatal dopamine neurotransmission, or hypodopaminergia, is a consequence of excessive ethanol use, and is hypothesized to be a critical component of alcoholism, driving alcohol intake in an attempt to restore dopamine levels; however, the neurochemical mechanisms involved in producing dopamine signaling deficiencies are unknown. Here we examined the specific dopaminergic adaptations induced by chronic ethanol self-administration that produce hypodopaminergia and may contribute to alcohol use disorders. Female rhesus macaques (3 controls, 5 drinkers) completed one year of daily (22 hr/day) voluntary ethanol self-administration. Animals were given ethanol access until 3.5-6.5 hours before *ex vivo* fast-scan cyclic voltammetry in post-mortem brain slices containing the nucleus

accumbens core was used to determine ethanol-induced alterations in dopamine terminal function including dopamine release and uptake kinetics as well as the ability of quinpirole (D2/D3 dopamine receptor agonist) and U50,488 (kappa-opioid receptor agonist) to inhibit dopamine release. Chronic ethanol drinking increased dopamine uptake rates, and uptake rates were positively correlated with lifetime ethanol intake. Further, the sensitivity of inhibitory D2/D3 dopamine autoreceptors and kappa-opioid receptors were also enhanced following drinking. Together, these factors likely converge to drive a hypodopaminergic state, characterized by an inability to mount an appropriate dopaminergic response to salient stimuli. Additionally, these data suggest that the dynorphin/kappa-opioid receptor system may be an efficacious target for pharmcotherapeutic interventions in the treatment of alcohol use disorders.

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Poster

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Kungliga och Hvitfeldtska Stiftelsen

Fredrik och Ingrid Thurings Stiftelse

Title: Repeated systemic administration of taurine influences the dopamine elevating properties of ethanol in the rat nucleus accumbens

Authors: *L. IVANOFF, L. ADERMARK, B. SÖDERPALM, M. ERICSON;
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Abstract: When trying to understand the development of addiction, a brain disease known to cause enormous suffering worldwide, much focus has been put on defining the mechanism by which the addictive drug increases dopamine in the mesolimbic dopamine system, which in turn may produce positive reinforcement. Ethanol, an addictive substance with a rich pharmacology, is known to modestly increase dopamine output in the nucleus accumbens (nAc), but the exact mechanism of action has been difficult to determine. We have previously demonstrated that

ethanol increases dopamine via a neuronal circuitry involving glycine receptors in the nAc and nicotinic acetylcholine receptors in the ventral tegmental area. We have also shown that in order for ethanol to increase dopamine in the nAc an initial enhancement of the extracellular levels of the endogenous amino acid taurine is required. Interestingly, intake of taurine has rapidly escalated over the last decade in several parts of the world due to consumption of so called energy drinks. This is interesting since taurine is known to activate both GABAA receptors and glycine receptors but few studies have investigated possible central effects of systemic taurine administration. Thus, by means of *in vivo* microdialysis in adult male Wistar rats we measured extracellular levels of dopamine, taurine, glycine and serine after systemic injections of vehicle, taurine (0.25 g/kg, ip), ethanol (2.5 g/kg, ip) or the combination of ethanol and taurine in rats pretreated with either taurine or saline for 15 days. We found that systemic administration of taurine increased extracellular taurine concentrations in the nAc and that subchronic treatment with taurine enhanced this increase. We also found that subchronic treatment with taurine blunted the ethanol-induced elevation of dopamine. Quantitative PCR analysis of tissue samples from the nAc as well as from other brain regions provided no evidence for altered mRNA expression of the taurine transporter or selected GABAA receptor subunits after subchronic taurine. We conclude that repeated systemic exposure to high amounts of taurine produces subtle alterations of neurochemical events previously suggested to be relevant for the reinforcing properties of ethanol.

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Poster

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DA035958

Title: Neurosteroids modulate ethanol effects on dopamine release in the nucleus accumbens via actions on GABA(A) receptors on VTA GABA neurons

Authors: ***T. J. WOODWARD**, D. M. HEDGES, A. C. NELSON, H. PARK, N. D. SCHILATY, S. C. STEFFENSEN;
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Abstract: Dopamine (DA) transmission in the mesolimbic reward system originating in the ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAc) is lowered during withdrawal from chronic ethanol exposure. Levels of neurosteroids with GABA(A) receptor-modulating properties are also lowered in the VTA by chronic ethanol, suggesting that select neurosteroids may be targets for restoring VTA DAergic function. The primary regulation of VTA DA neuron excitability is mediated by GABA(A)-mediated inhibition, presumably from local circuit VTA GABA neurons. We have shown in multiple reports that VTA GABA neurons are inhibited by acute ethanol and become hyperexcitable during withdrawal from chronic ethanol, presumably due to a switch in GABA(A) receptor function on VTA GABA neurons. VTA GABA neurons inhibit DA release either through local circuit inhibition or via projections to DA terminals in the NAc. The goal of this study was to determine the involvement of GABA(A) modulating neurosteroids in VTA GABA neuron excitability and in DA neurotransmission in the mesolimbic pathway. Using fast scan cyclic voltammetry (FSCV), we performed experiments on brain slices in the NAc by superfusing various GABA(A)R-modulating neurosteroids - allopregnanolone, DHEAS, and estrone sulfate. We also tested Trilostane, which enhances the endogenous expression of DHEAS via block of 3β-hydroxysteroid dehydrogenase. DHEAS (20 μM) enhanced DA release in the NAc by 20 %, while allopregnanolone, estrone sulfate, and Trilostane did not significantly alter DA release. Ethanol (20 - 160 mM, IC50 = 80 mM) reduced DA release in wild type (WT) mice. Superfusion of DHEAS (20 μM) significantly attenuated ethanol inhibition of DA release. However, the other neurosteroids did not significantly alter ethanol inhibition of DA release. In electrophysiological single-unit studies *in vivo*, DHEAS markedly enhanced VTA GABA neuron firing rate. Experiments are in progress to further evaluate the effects of the GABA(A) receptor-modulating neurosteroids on VTA and NAc neuron firing rate as well as GABA(A) receptor-mediated synaptic transmission to VTA GABA neurons.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

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Title: The lateral septum and its involvement in ethanol-induced dopamine elevation

Authors: *M. ERICSON, J. MORUD, S. JONSSON, B. SÖDERPALM;
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Abstract: Alcohol and other addictive drugs share the ability to increase extracellular dopamine levels in the nucleus accumbens (nAc), an important node of the brain reward system. Since this specific increase in neurotransmitter levels has been linked to the positive and reinforcing properties of addictive drugs, and dopamine elevation is one of the few common effects produced by these drugs, much focus have been aimed at understanding the mechanism(s) underlying the extracellular increase of dopamine. Ethanol is a small molecule with a wide range of effects in the brain and several hypotheses have been put forward regarding its dopamine elevating properties. We have, in a long series of studies, tried to pinpoint how ethanol increases extracellular dopamine levels in nAc and have suggested that ethanol activates a neuronal circuitry involving glycine receptors in the nAc, and, secondarily, nicotinic acetylcholine receptors in the ventral tegmental area (VTA). The results of the present study include the lateral septum (LS), a brain region previously associated with ethanol-related behaviors, in the neuronal circuitry mediating the dopamine increasing effect of ethanol. By means of qPCR, retrograde tracing and *in vivo* microdialysis in Wistar rats we found that neurons projecting from LS to either VTA or nAc are GABAergic and that they likely express glycine receptors. Administration of tetrodotoxin in the LS slightly but significantly decreased basal dopamine levels in the nAc and completely prevented ethanol but not nicotine from increasing extracellular accumbal dopamine levels. Thus, we suggest that the LS participate in modulating basal dopamine levels in the nAc as well as the ethanol-induced dopamine elevation in the same area. The LS may thus be involved in mediating the reinforcing effects of ethanol, even though its involvement in ethanol intake or alcohol addiction remains to be established.

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Poster

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Title: Key role of dopamine signaling in spine pruning and LTD formation in ethanol dependence

Authors: G. MULAS[1], G. TALANI[3], E. SANNA[1], M. DIANA[4], *S. SPIGA[2];
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Abstract: Alcoholism involves serious and persistent changes in the brain, including memory impairment and cognitive deficits. Changes in dopaminergic signaling are likely to contribute to synaptic dysfunctions that quickly occur from suddenly ceasing the use of alcohol after chronic ingestion. Here we show the effects of L-Dopa treatment on the reduction of long-thin spines and LTD formation produced by ethanol withdrawal (Spiga et al., 2014) in medium sized neurons of the shell of the nucleus accumbens (NAcc). Confocal microscopy and modified Golgi Cox staining analysis revealed a unique remodeling of the long thin spines selectively after 12 h of ethanol suspension. Reduced, long thin spine density observed during withdrawal promptly returns to control values as a result of a single injection of L-Dopa 1h before sacrifice. Further, whole cell patch clamp recordings of eEPSP from MSNs of NAcc shell revealed that, as previously reported, 12h Wdl from chronic EtOH treatment, are associated with an hampered LTD formation on glutamatergic excitatory synapses. Interestingly, the marked effect induced by Wdl is reverted by L-DOPA injection (6mgKg of body weight) 1h before sacrifice, as well as 10 μM dopamine (DA) perfusion in the slice from Wdl animals, 5 or 30 min before the low frequency stimulation (LFS) protocol given to induce LTD induction. In addition, the effect induced by DA was antagonized by the D1 selective antagonist SCH23390.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Title: Brain morphology, nutritional and behavioral evaluation of rats exposed to chronic alcohol intake

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Abstract: Alcoholism has a tremendous impact on morphology and functioning of the nervous system. Chronic ethanol intake is a common cause of addiction with multiple interferences. Because alcoholism has its widespread multifactor interference, the present study aimed at analyzing brain morphology, nutritional and behavioral alterations resulting from chronic alcohol exposure in rats. This work occurred under the approval of the ethics committee and all procedures were conducted to minimize suffering of animals. Adult young male wistar rats (n=16) were housed in standard cages and provided with balanced food ad libitum. A control group (CRT, n=8) provided with water and food ad libitum was compared with a group of chronic alcohol drinkers (ALC) for 4 months. ALC received bottles with a traditional and largely consumed alcoholic beverage in Brazil, known as cachaça. Crescent concentrations of cachaça at 5, 10, 15 and 20% were administered for a week each. After this adaptation period, ALC received cachaça, at 25% in drinking water for 4 months. During experimental stages the specimens were submitted to hydric restriction to approximately 80% of their weight ad libitum, aiming the reinforcement effect of water. All animals were submitted to activities that involve prior stimuli of discriminative tasks and sensitivity to their proper behavior. Chambers equipped with a drinking water bar that releases 0.05ml each touch were used for operating conditioning tasks. An additional floor was used to create the open field environment. Animals passed through alcoholism induction period, monitoring of food intake and body weight, behavioral tests and brain analysis. Preliminary results have shown body weight mean initial (401.6 and 407.6g) and final (496.3g and 535.6g) for CRT and CAC, respectively. Histomorphological analysis revealed sings of defragmented and degenerated tissue in ALC brains, with special effect in the cortical layers. Periodic observations and recording for behavioral parameters showed hyperactivity in ALC, compared to CRT. Bearing all this in mind, it may be concluded that chronic condition of cachaça's intake can damage specific areas of the thelencephalon, associated with body weight changes, food consumption and behavioral alterations. These findings were consistent with preview studies on alcoholism but further investigations regarding the use of cachaça are encouraged to elucidate other specific effects of this model.

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Poster

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P50 AA07611

Title: Genomic signatures of alcohol preference in the HAD1/LAD1 and HAD2/LAD2 replicate rat models

Authors: *C.-L. LO[1], W. M. MUIR[2], A. C. LOSSIE[5], H. J. EDENBERG[3], T. LIANG[2], Y. LIU[4], F. C. ZHOU[1];
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Abstract: Familial drinking is a key factor in the alcoholism. However, the genetic contribution is not clear. A number of GWAS studies in human tissue were conducted, but due to limitation and confounding of genetic and environmental factors, these have not provided a clear view on genomic association. To avoid these confounding factors, we use a replicated bi-directionally selected animal model, the high alcohol drinking (HAD) rat and low alcohol drinking (LAD) rat which has been shown to fit every criteria of alcoholism for high resolution sequencing studies. To reduce chance events, replicated line HAD1/LAD1 and HAD2/LAD2 were studied. Spleen DNA of 10 animals from each line, a total of 40 animals were sequenced at 4.8X coverage of sequence. After quality controlling and removal of false positive, over 5.5 millions single nucleotide polymorphisms (SNPs) were screened between HAD and LAD in both replicates. Furthermore, we identified significant SNPs and classified them by gene regions (15KB promoter, intron, exon, exon/intron junction). While most of the regions were partially confounded due to LD, most of the regions were in promoters and intronic areas and few were in the exons. These results suggest that SNPs in regulatory regions contribute greater to drinking behavior than those in the coding region. Many of those SNP involved genes are associated with potential alcohol preference and neuronal function, such as ion channels (Kcnk1, Scn5a, Cacna1h, etc.), and transmitter/synatic receptors (Gria3, Grip1, Chrna10, Syngap1, etc.). In addition, several genes have been previously reported in separate human GWAS studies, including Shank2, Syt1, Tnxb, Adcy3, Ssbp3, and Sh3bp5. IPA pathway analysis reveals canonical pathways controlling neuronal plasticity and LTP in CREB signaling pathway, PPARa/RXRa activation, and Axon guidance signaling may be affected. Overall, we presented unique finding in signature of selection in response to drinking behavior. Our finding implicates potential biomarkers for further analysis of genomic contribution. Better understanding of the genomic-environmental interactions will enhance the understanding of the mechanism and

diversity of alcoholism, for prevention, diagnosis, and treatment. Supported by NIH AA016698 and P50 AA07611.

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Poster

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F30 AA021637

Title: Alcohol-induced changes in cannabinoid modulation of noradrenergic neurons

Authors: ***R. WYROFSKY**[1], B. A. S. REYES[1], T. RETSON[2], J. HOEK[3], E. J. VAN BOCKSTAELE[1];
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Abstract: Alcohol use disorders (AUDs) affect approximately 8.5% of American adults, and while remission is achievable, longitudinal data indicate that 60-90% of alcoholics will relapse within their first four years of abstinence. Moreover, sex differences related to alcohol's negative effects are emerging. For example, chronic alcohol exposure causes increased negative physiological effects on bodily organs and increases feed-forward activation of the hypothalamo-pituitary-adrenal axis in females when compared to males. One major symptom of alcohol withdrawal is increased stress-induced anxiety, which can often lead to relapse in recovering alcoholics. The locus coeruleus (LC) provides the sole source of norepinephrine (NE) to the frontal cortex and high levels of cortical NE have been implicated in the pathophysiology of stress-related psychiatric disorders. Via corticotropin-releasing factor (CRF) neurotransmission, stress exposure activates LC neurons, increasing the release of NE in forebrain targets. Chronic alcohol exposure alters CRF activation of LC-NE neurons and subsequently impacts NE release. The endocannabinoid (ecB) system regulates neurotransmitter release, and emerging studies suggest targeting the eCB system may influence the development of anxiety and stress-induced

psychiatric disorders. Taken together, actions of CRF and eCBs may converge in the LC to modulate stress-induced anxiety responses. However, the effect of alcohol on this interaction remains unknown. Here, we investigated the effect of repeated alcohol administration on cannabinoid type 1 receptor (CB1r) in the LC using Western blot analysis. Male and female Sprague-Dawley rats were match-pair fed a calorically equivalent liquid diet containing 36% of calories as ethanol for 14 days to induce an ethanol-addicted state. Alcohol treated females demonstrated a significant decrease in CB1r levels when compared to control and male counterparts ($p<0.05$). We also expanded on prior results showing co-existence of CB1r and CRF by examining whether CB1r/CRF afferents were excitatory or inhibitory in nature. Triple immunofluorescence microscopy showed that CB1r/CRF afferents expressed either the vesicular glutamate transporter or glutamic acid decarboxylase in the LC. These studies suggest that alcohol causes a greater impact on the eCB system in females compared to males in the stress-integrative LC and that CB1r can regulate CRF release in afferents that express excitatory and inhibitory amino acids.

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Poster

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Support: NSF via the S-STEM Program Award No.DUE-1153832

NIAAA R15AA020996

NIMHD 2G12MD007592

Title: Neuromodulatory mechanism underlying ethanol-induced behavioral disinhibition

Authors: *G. P. ARANDA[1], K.-A. HAN[2], J. LIM[2], P. SABANDAL[2], P. D. EVANS[3]; [1]Biosci. Building, Univ. of Texas At El Paso, El Paso, TX; [2]Dept. of Biol. Sci., Univ. of Texas at El Paso, El Paso, TX; [3]The Babraham Inst., Cambridge, United Kingdom

Abstract: Alcohol exerts numerous effects on behavior through its interaction with diverse membrane and signaling molecules and effector cells. The effects include lack of motor control, behavioral disinhibition, tolerance, sensitization and addiction. In particular, behavioral

sensitization to alcohol's disinhibiting effects is strongly associated with binge drinking and alcoholism. Dopamine neurotransmitter plays a central role in ethanol-induced behavioral disinhibition and sensitization. However, identifying the neural sites that individual dopamine receptors function and underlying cellular mechanisms needs to be resolved. For this task, we have used *Drosophila* melanogaster as a model organism, which allows us to readily track and manipulate important sites of molecular and neural functions. The focus of this study is the D1-like receptor DopEcR, an insect G-protein coupled receptor that can bind to both dopamine and steroid hormone ecdysone. We found that DopEcR deficient (der) male flies show abnormal disinhibited courtship and decreased synaptic plasticity upon recurrent alcohol exposures. The der mutant's courtship phenotype was fully restored by expressing DopEcR during adulthood. This indicates a physiological, rather than developmental, role of DopEcR in ethanol induced disinhibition and sensitization. We are currently investigating the neural sites that DopEcR functions to regulate behavioral disinhibition and sensitization. We are also conducting experiments to clarify the relative contributions of dopamine and ecdysone to the phenotypes. This study will clarify key *in vivo* functions for a novel insect G-Protein coupled dopamine/steroid receptor and signaling molecules in alcohol-associated behaviors. This research was supported by the National Science Foundation via the S-STEM Program Award No. NSF DUE-1153832, the NIAAA R15AA020996 and NIMHD 2G12MD007592 grants.

Disclosures: G.P. Aranda: None. **K. Han:** None. **J. Lim:** None. **P. Sabandal:** None. **P.D. Evans:** None.

Poster

695. Alcohol: Neural Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 695.19/N30

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R00AA020537

Title: The impact of chronic alcohol exposure on fear-related memories: a role for mGluR5

Authors: J. MCGONIGAL, H. HAUN, *J. T. GASS;
Neurosciences, Med. Univ. South Carolina, Charleston, SC

Abstract: Despite the high rates of comorbidity between alcohol use disorder (AUD) and post traumatic stress disorder (PTSD) there is a substantial gap in our knowledge concerning how these disorders interact to cause significant deficits in behavior and cognition. For example, how

does chronic alcohol abuse affect PTSD-related fear memories? Also, how does PTSD drive subsequent alcohol abuse? This set of studies examined the impact of chronic intermittent ethanol (CIE) exposure on the extinction of fear behavior and, conversely, how exposure to fear-related cues influences subsequent ethanol consumption. Male Wistar rats were first exposed to a rodent model of PTSD in which a tone (CS) was associated with mild foot shock. After fear conditioning, rats were assigned to either CIE vapor exposure or a control group that received air exposure for two weeks. Prior to the ethanol/air exposures, each rat was briefly placed in the fear environment to activate the fear memory. Rats were then exposed to a daily extinction training regimen (10 tone presentations, but no shocks). A "contextual" recall test followed two days after the final day of extinction. Prior to each extinction session, rats were treated with either the mGluR5 PAM CDPPB (30 mg/kg) or vehicle solution. Compared to air-exposed controls, rats exposed to CIE showed deficits in the extinction learning of fear behavior and in extinction memory consolidation and recall. Treatment with CDPPB, however, attenuated the extinction deficits observed in CIE-exposed rats. In a separate experiment, male Wistar rats were first conditioned to associate a tone with foot shock and then exposed to CIE. All rats were then placed in their home cage and exposed to a two-bottle intermittent access choice paradigm. They received access to a 20% ethanol solution for a period of 24 hours in their home cage three days per week. Water was freely available. After 3 weeks one group was exposed to a single session of fear extinction (10 tone presentations). The control group was not exposed to the CS. All rats were placed back in their home cage and again given access to ethanol and water for 24 hours. The results indicated that a one-time session of repeated exposures (10) to the fear CS lead to a significant increase in ethanol consumption compared to the group not exposed to fear cues. Together, these studies suggest that an interaction of CIE and fear conditioning leads to altered extinction behavior and ethanol consumption while modulation of mGluR5 may serve as a potential treatment mechanism. Studies are currently being conducted using optogenetics to isolate specific neurocircuitry involved in these AUD/PTSD behavioral changes.

Disclosures: J. McGonigal: None. **H. Haun:** None. **J.T. Gass:** None.

Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIAAA R03AA022479

Title: Ketamine prevention of alcohol-induced depressive-like behavior is pharmacokinetic-independent

Authors: *L. AKINFIRESOYE, O. KALEJAIYE, Y. TIZABI;
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Abstract: Treating the depression associated with alcoholism is key to improving the success rate of treating alcoholics and preventing relapse. Hence, antidepressants may be of particular therapeutic potential in such cases. However, current antidepressants including serotonin reuptake inhibitors can take several weeks to become effective and are invariably associated with side effects. Ketamine, and NMDA receptor antagonist, commonly uses as a dissociative anesthetic, has been shown to have fast and durable action as an antidepressant in sub-anesthetic doses. We have preliminary data indicating that a very low dose of ketamine given daily prior to alcohol administration can normalize alcohol-induced behavioral impairments reflective of depressive-like behavior in male Wistar rats. Thus, ketamine (2 mg/kg i.p.) blocked the exaggerated immobility in the forced swim test (reflective of helplessness) and decrease in sucrose intake (reflective of anhedonia) induced by daily alcohol administration of 1 g/kg for 7 days. In this study, we sought to determine whether ketamine's effect may also be due to its pharmacokinetic interactions with alcohol, i.e., whether ketamine may reduce the blood alcohol concentration. For this purpose we obtained tail blood from rats 30 min prior to alcohol administration (1 g/kg i.p.) and every 30 min (up to 120 min) after alcohol and alcohol + ketamine (2 mg/kg i.p. 30 min prior to alcohol). Blood alcohol concentrations peaked at 30 min post alcohol administration (approximately 89 mg%) and was back to normal levels at 2 h. Ketamine treatment did not affect blood alcohol levels at any time point. These data suggest that ketamine's antidepressant effects in alcohol-induced depression are independent of its pharmacokinetic interactions with alcohol. Current studies are underway to determine the neurobiological substrates associated with alcohol-induced depression and ketamine's mechanism(s) of action. Supported by: NIH/NIAAA R03AA022479

Disclosures: L. Akinfiresoye: None. **O. Kalejaiye:** None. **Y. Tizabi:** None.

Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: R24-GM092842 (CVM)

F32- NS062609 (CRO)

P60AA010760 through the Portland Alcohol Research Center (PARC)

Title: Alcohol affects vocal learning and activation of underlying basal ganglia of zebra finches

Authors: *C. R. OLSON, D. C. OWEN, A. E. RYABININ, C. V. MELLO;
Dept. of Behavioral Neurosci., OHSU, Portland, OR

Abstract: Speech has long been known to be altered by alcohol, yet studies of the mechanistic role of alcohol on the production or acquisition of learned vocal patterns have languished due to the lack of an appropriate model system. We recently showed that zebra finches, a representative songbird that serves as the primary model to study the neurobiology of vocal learning and production, will self-administer alcohol to high blood ethanol levels (BECs; 50-90 mg/dl), resulting in acute effects on the acoustic features of their learned songs. Song learning resembles human vocal learning in that male finches acquire their songs within a critical juvenile period during which they establish a tutor song auditory memory, present immature vocalizations akin to infant babbling, and learn to imitate the tutor song over a prolonged period of sensorimotor practice. Here we describe the effects of alcohol on song learning. Juveniles were provided free access to 3.5% alcohol during the song learning period (from 45 - 125 days old), while control siblings received only water. Singing was recorded weekly during alcohol-free conditions, until adulthood. To evaluate the effects of alcohol on singing behavior we analyzed song stereotypy and the similarity of songs in experimental and control groups compared to tutors. Alcohol increased stereotypy of undirected and directed song, and these effects were detectable within 10 days after the initial exposure to alcohol. The similarity of juvenile song to tutor song was diminished in the alcohol group, suggesting that these juveniles were impaired either in recalling or imitating the tutor template. We also note increased horizontal vocal learning among alcohol-treated finches, as they were apt to learn some vocalizations from cage mates. In an assessment of the mechanism that underlies these behavioral effects, 70 d male finches were given 6.5% alcohol (or water only for controls) and sacrificed 30 minutes following a period of intense singing. In-situ hybridization revealed that alcohol decreased singing-induced ZENK (egr-1) expression in Area X, a basal ganglia nucleus crucial for song learning. ZENK expression in HVC, a nucleus responsible for the initiation of vocal-motor output, was less affected. We suggest that alcohol affects song development in juveniles by inhibiting activity in a circuit required for vocal motor plasticity, limiting the birds' ability to learn tutor song. The finch offers a powerful model to examine the effects of alcohol on brain development and vocal production, and can aid in the understanding of how alcohol affects cognitive development, particularly as it pertains to the acquisition of speech and language skills.

Disclosures: **C.R. Olson:** None. **D.C. Owen:** None. **A.E. Ryabinin:** None. **C.V. Mello:** None.

Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIMHD Grant 2G12MD007592

NIH/NIAAA Grant R15AA020996

NIH/NIAAA Grant R15AA020996-01S1

Title: Ethanol-induced behavioral disinhibition

Authors: *I. MERCADO, P. SABANDAL, J. BURCIAGA, K.-A. HAN;
Univ. of Texas at El Paso, El Paso, TX

Abstract: Dopamine is a major neuromodulator regulating cognition, reward, movement control, learning and memory. Alcohol consumption affects these cognitive and behavioral processes. To elucidate the underlying mechanisms, we used *Drosophila* melanogaster as an animal model since it has dopamine synthesizing enzymes, receptors and transporter homologous to those in humans. Flypub and FlyTracker assays are used to monitor disinhibited sexual and motor behaviors. In this study we investigated the dopamine receptors for ethanol-induced hyperactivity, sedation, tolerance, courtship disinhibition, and sensitization. We found that the flies deficient in or overexpressing D2 receptor (dD2R) showed altered ethanol sensitivity and courtship disinhibition whereas D1 (dDA1) and D5 (DAMB) receptor mutants displayed enhanced courtship disinhibition with normal sensitivity and tolerance. We are currently examining the neural pathways underlying these behavioral changes. This work was supported by the NIH grants: NIMHD 2G12MD007592, NIAAA R15AA020996 and NIAAA R15AA020996-01S1.

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Poster

695. Alcohol: Neural Mechanisms

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH RO1 AA15150

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Fondecyt 1131004

Title: Inhibition of the ethanol-induced potentiation of glycine receptors by small molecules that interfere with Gβγ binding

Authors: *L. S. SAN MARTIN[1], F. E. CERDA[2], C. JIN[3], L. G. AGUAYO[2], J. L. GUZMAN[2]; [2]Dept. de Fisiología, [1]Univ. De Concepcion, Concepcion, Chile; [3]Ctr. for Organic and Medicinal Chem., Res. Triangle Inst., North Carolina, NC

Abstract: Ethanol enhances the glycine receptor (GlyR), a ligand-gated ion channel (LGIC) critical for inhibitory neurotransmission in brain stem and spinal cord and possibly associated to reduced motor coordination and respiratory rhythm during acute intoxication. We previously found that peptides interfered with the binding site controlling the interaction between Gβγ and GlyR, and consequently with the ethanol modulation of the receptor. We performed a virtual screening study using a library of small molecules that allowed us to identify a subset of potential pharmacological compounds capable of binding to this binding site. At a concentration of 200 μM, M554 (an indole derivative) inhibited ethanol potentiation from 48 ± 3% in control to 30 ± 2% with 100 mM of ethanol, whereas 200 μM of M890 (a urea derivative) decreased ethanol potentiation to 26 ± 3%. The molecules did not interfere significantly with Gβγ activation of GIRK channels expressed in HEK293 cells. Interestingly, the potentiation of the decay time constant of synaptic GlyR currents in spinal cord neurons produced by ethanol was inhibited by M554 and M890. Thereafter, when we assayed the effect of M554 (200 mg/kg) on the loss of righting reflex (LORR) in presence of 3.5 g/kg of ethanol in C57BL/6J mice, we found that the mice injected with M554 recovered their reflex capacity faster than control mice (45.95 ± 3.6 min for control and 36.35 ± 2.2 min for M554 injected mice). Our results indicate that these small molecules have synaptic effects in an ethanol potentiation model of glycinergic transmission and animal behavior. This data will provide us with information that might help in the design of therapeutic molecules useful in the treatment of acute alcohol intoxication and abuse complications.

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Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: P/PIFI-2013-23MSU0140Z-09

Title: Quantitative EEG differences between subjects with hazardous alcohol consumption and subjects with alcohol dependence

Authors: *L. NUÑEZ-JARAMILLO[1], W. V. HERRERA-MORALES[2], L. RAMÍREZ-LUGO[3], J. V. REYES-LÓPEZ[4], E. SANTIAGO-RODRÍGUEZ[5];
[1]División De Ciencias De La Salud. Univ. De Quintana Roo, Chetumal, Quintana Roo, Mexico; [2]División de Ciencias de la Salud, Univ. de Quintana Roo, Chetumal, Quintana Roo, Mexico; [3]Inst. de Fisiología Celular, Univ. Nacional Autónoma de México, México D.F., Mexico; [4]Facultad de Medicina, Univ. Autónoma de Querétaro, Querétaro, Mexico; [5]4Diagnóstico, Tratamiento e Investigación Neurológica, S.C., Querétaro, Mexico

Abstract: Harmful alcohol use is among the top five risk factors for disease, disability and death worldwide. Hazardous alcohol consumption is a pattern of alcohol consumption that increases the risk of harmful consequences for the user or others, and has been linked with risky behaviors, accidents and injuries. It is noteworthy that hazardous alcohol consumption does not necessarily implies alcohol dependence. While there is a large body of research on the neurophysiological correlates of alcohol dependence there is little information so far about neurophysiological correlates of hazardous alcohol consumption. It has been reported that subjects with hazardous alcohol consumption but not alcohol dependence present increased beta absolute power at centro-parietal region, and decreased beta mean frequency (MF) in frontal centro-parietal regions. While increased beta absolute power has been already reported in alcohol dependent subjects, no report has addressed beta MF in these subjects, thus it is not yet known whether this decrease in beta MF is a feature correlated specifically with hazardous alcohol consumption or is also related with alcohol dependence. Herein we performed qEEG analysis of beta MF in subjects with alcohol dependence and in subjects with hazardous alcohol consumption but not alcohol dependence. We found decreased Beta MF in subjects with hazardous alcohol consumption but not in subjects with alcohol dependence. Our results suggest that differences exist in the neurophysiological correlates of alcohol dependence and hazardous alcohol consumption, supporting the hypothesis that there are differences in the neurophysiological mechanisms underlying these two conditions

Disclosures: L. Nuñez-Jaramillo: None. **W.V. Herrera-Morales:** None. **L. Ramírez-Lugo:** None. **J.V. Reyes-López:** None. **E. Santiago-Rodríguez:** None.

Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIDA #R01AA017656

F31AA022567

Title: Assessing gender differences in nicotinic acetylcholine receptor contributions to ethanol consumption and reward

Authors: *M. G. DERNER, A. V. SACINO, P. D. GARDNER, A. R. TAPPER;
BNRI Room 144, Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Alcoholism is the third leading cause of preventable mortality in the world highlighting the need for a better understanding of the mechanism underlying the rewarding properties of alcohol. In addition, gender influences alcohol consumption and dependence, with men twice as likely to binge drink compared to women. Previous studies in rodent models indicate that cholinergic signaling via nicotinic acetylcholine receptors (nAChRs) containing the alpha4 subunit (α4* nAChRs) contributes to alcohol binge drinking and reward in C57BL/6J mice. Specifically, we have shown that male alpha4 nAChR subunit knock-out mice (KO) consume less ethanol in the "drinking in the dark" (DID) assay compared to wild-type (WT) littermates and fail to condition a place preference to 2.0 g/kg ethanol in the conditioned place preference (CPP) paradigm. To determine if similar deficits are observed in female α4 KO mice compared to WT, we measured ethanol consumption in the DID assay and ethanol place preference in these animals. During DID, mice were single housed, placed on a reverse light-dark cycle (lights on at 7PM, off at 7AM), and allowed to acclimate for two weeks. Following this period, mice were given a single bottle of ethanol, in place of their water bottle, two hours into the dark cycle for two hours. Bottles were weighed before and after each drinking session and ethanol consumption was calculated. This was done four days a week, and each week the percent ethanol was increased (2, 5, 10, 20%). CPP was done using a three-chamber apparatus with a 30-min habituation on day 1, two training periods (one for saline, one for ethanol) separated by 6 h on days 2-5, and a 30-min test on day 6. Unlike their male counterparts, female α4 KO mice exhibited similar ethanol consumption compared to WT. No differences in control sucrose or saccharin consumption were observed between female WT and α4 KO mice. In the CPP assay, 0.5 g/kg ethanol, but not 2.0 g/kg ethanol, conditioned a place preference in WT females, whereas 2.0 g/kg ethanol conditioned a place preference in α4 KO females, suggesting a shift to the right in the ethanol dose response relationship in KO females. These data suggest

different roles for α4* nAChRs in males and females with respect to ethanol behaviors, and also indicate a potential difference in sensitivity to ethanol between the two genders.

Disclosures: **M.G. Derner:** None. **A.V. Sacino:** None. **P.D. Gardner:** None. **A.R. Tapper:** None.

Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA018779

Title: Adenosine mediated hippocampal-accumbal neuroproteome alterations in equilibrative nucleoside transporter 1 null mice

Authors: ***A. OLIVEROS**[1], S. CHOI[2], D. HINTON[2], C. VADNIE[2], D.-S. CHOI[2];
[1]Mol. Pharmacol. & Exptl. Therapeut., Mayo Clin., Rochester, MN; [2]Mayo Clin., rochester, MN

Abstract: The neural circuitry consisting of the dorsal hippocampus and nucleus accumbens contributes to cue-induced learning and ethanol-seeking behaviors in mice. Adenosine plays an essential role in hippocampal and accumbal regulated addictive behaviors. Mice lacking the ethanol-sensitive, adenosine equilibrative nucleoside transporter (ENT1-/-) show increased ethanol drinking, thus we investigated the proteome profile of the dorsal hippocampus and nucleus accumbens of ENT1 -/- and WT mice using label-free quantitative proteomics. We identified 1,196 proteins overlapping between the two regions, 670 mapping exclusively to the dorsal hippocampus, and 2,436 to the nucleus accumbens. Ingenuity Pathway Analysis (IPA) detected region specific significant changes in protein expression that significantly associated with biological processes, including molecular transport, basal ganglia disorders, glutamate degradation, and GABA receptor signaling. Western blot validation of our proteomic analysis confirmed a significant decrease in expression of the SNARE protein VAMP1 and glutamate receptor mGLUR3 in the dHPC of ENT1-/- mice, both of which are associated with molecular transport and presynaptic glutamate release. Similarly we detected a significant decrease in expression of the oxidative phosphorylation Complex-I protein NDUFS2 and a significant increase in the Complex-IV protein COX6c, both of which were significantly associated via IPA with mitochondrial dysfunction. Our findings suggest an important role for adenosine regulation

in the hippocampal-accumbal circuit, which may reveal novel aspects of adenosine signaling in alcohol use disorders.

Disclosures: **A. Oliveros:** None. **S. Choi:** None. **D. Hinton:** None. **C. vadnie:** None. **D. Choi:** None.

Poster

695. Alcohol: Neural Mechanisms

Location: Hall A

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Topic: C.17. Drugs of Abuse and Addiction

Support: University of Palermo

Title: Intermittent- vs continuous alcohol access in female rats: effects on deprivation phenotype and maternal behavior as a consequence of the drinking pattern

Authors: ***A. BRANCATO**[1,2], C. VITA[1], F. PLESCIA[1], C. CANNIZZARO[1];
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Abstract: In male rats, the intermittent alcohol access paradigm produces relevant and specific consequences on neuronal activity and behavior, with respect to traditional free-access paradigms (Carnicella et al., 2014). In order to explore gender-related effects, this study aimed at assessing the consequences of two different patterns of alcohol self-administration on peculiar feminine behavioral repertoire, such as deprivation phenotype and maternal care. Animals underwent long-term, home cage, two-bottle "alcohol (20% v/v) or water" choice regimen, with continuous (7 days a week) or intermittent (3 days a week) access, and were tested for alcohol intake and preference. During acute deprivation, they were tested for behavioral reactivity in the open field; anxiety-like behavior in the traditional- and fear-potentiated elevated plus maze; novelty-induced exploration and recognition memory in the novel object recognition test; response to natural reward in saccharin preference test; depressive-like behavior in the forced-swim test. Animals were alcohol-deprived during mating and resumed self-administration from late gestation and throughout lactation. Home-cage undisturbed maternal behavior was assessed until weaning. Results show that rats exposed to intermittent access paradigm displayed higher alcohol intake and preference with respect to rats with continuous access ($p<0.001$). During deprivation, rats exposed to intermittent access manifested reduced response to fear, novelty and reward ($p<0.001$), and depressive-like behavior ($p<0.001$), whereas rats exposed to continuous access displayed a prominent anxiety-like behavior ($p<0.001$). Moreover, alcohol drinking

decreased nursing and overall maternal behaviour; the most detrimental consequences were observed in dams with intermittent alcohol access ($p<0.001$). In conclusion, long-term alcohol drinking induces profound alterations in the neuroadaptive systems underlying affectivity and reward, leading to alcohol-deprivation phenotypes and disruption of maternal-care behaviour in a drinking pattern-related manner.

Disclosures: **A. Brancato:** None. **C. Vita:** None. **F. Plescia:** None. **C. Cannizzaro:** None.

Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Title: ALDH2 deficiency increases the sensitivity to cannabis as well as ethanol-induced hypothermia and motor impairment in mice

Authors: ***X. LIU**, L. WAN, G. LUO, D. LOVINGER, L. ZHANG;
Natl. Inst. of Hlth., NIAAA/NIH, Bethesda, MD

Abstract: Alcohol and marijuana are among the most widely used and abused substances in the western world. There is strong clinical evidence showing that concurrent use of both drugs can synergistically suppress brain function. In the human body, alcohol is rapidly converted into acetaldehyde by alcohol dehydrogenase (ADH). This toxic aldehyde is mainly metabolized by a key enzyme, aldehyde dehydrogenase 2 (ALDH2). ALDH2 deficiency, caused by a point-mutation, frequently occurs in the human population, with allele frequencies reaching up to around 40%, depending on ethnic background. This deficiency in ALDH2 is associated with the alcohol flushing reaction and cancer. Here, we ask if ALDH2 deficiency can alter motor impairment and hypothermia induced by concurrent treatment with a low dose ethanol and the major psychoactive component of marijuana, delta 9-tetrahydrocannabinol (THC) using rotarod performance and core body temperature measurements in ADH1, ALDH2 knockout mice and their wild type littermates. THC alone at low doses from 0.1 mg/kg to 5 mg/kg, i.p. or 1-5 μg i.c.v. did not significantly alter rotarod performance and body temperature in the mutant and wild type mice. Pretreatment with THC synergistically enhanced EtOH (1g/kg, i.p.)-induced motor impairment and hypothermia in mutant mice and their wildtype (WT) littermates. The impairment in ALDH2 deficient mice was significantly stronger than in WT and ADH1 deficient mice. Moreover, THC when given 30 min after EtOH administration induced synergistic effects on motor deficiency and hypothermia only in ALDH2 deficient mice but not in WT or ADH1

deficient mice. These findings suggest that cannabis may interact with acetaldehyde to produce synergistic behavioral effects *in vivo*. Supported by the NIAAA Division of Intramural Clinical and Biological Research and AA017875.

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Poster

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DA033533 (N.S)

DA037294 (N.S.)

AA023183 (N.S.)

Title: History of drug intake leads to compulsive appetite via disruption in non-homeostatic control of food intake

Authors: *A. LAQUE[1], N. SUTO[1], Y. HAO[1], A. MATZEU[1], G. DE GUGLIELMO[1], T. KERR[1], R. MARTIN-FARDON[1], T. JHOU[2], R. C. RITTER[3], F. WEISS[1];
[1]Mol. and Cell. Neurosci., The Scripps Res. Institue, LA Jolla, CA; [2]Med. Univ. of South Carolina, Charleston, SC; [3]Washington State Univ., Pullman, WA

Abstract: While the 'food addiction' model of pathological overeating has been popularized, fundamental shortcomings of this nosology still remain to be addressed. Nevertheless, striking similarities in behavioral manifestations and their neurobiological underpinnings exist between substance dependence and eating disorders. We hypothesized that a history of extensive drug or alcohol intake known to result in 1) addiction-like drug motivation and 2) addiction-linked brain changes would result in similar addiction-like motivation for food in rats. Initially, different groups of rats were trained to self-administer cocaine or saccharin (controls). All rats were then given opportunities to self-administer sweetened condensed milk (SCM). The history of cocaine

intake led to addiction-like SCM seeking and taking, marked by heightened resistance to 1) increased workload, 2) extinction, and 3) adverse consequences. However, a history of extensive cocaine intake failed to alter subsequent 1) bodyweight gain, 2) ad libitum chow intake 3) and, interestingly, ad libitum consumption of a highly palatable (high fat/ high sugar) diet. Because the addiction-triggering history of extensive drug intake is insufficient to induce overeating per se, the addiction-like motivation for SCM or 'compulsive appetite' observed in these rats is likely due to dysregulation of non-homeostatic (non-metabolic) rather than homeostatic (metabolic) control of food intake. Consistent with this hypothesis, two additional groups of rats with either a cocaine or alcohol history exhibited heightened resistance to an electric footshock paired with the delivery of a non-caloric saccharin reward. Moreover, a history of cocaine intake induced functional upregulation of group II metabotropic receptors (mGluR2/3) in medial prefrontal cortex (mPFC) and amygdala; brain sites implicated in non-homeostatic control of food intake. These receptors negatively modulate neural excitability via inhibitory Gi proteins and, thus, may contribute to impaired functional connectivity between mPFC and amygdala observed in drug addicts. Together, the nosology of addiction is most applicable to certain phenotypes of eating disorders characterized by compulsive appetite, such as binge-eating disorder and bulimia nervosa, rather than pathological overeating in general. Neurobiological irregularities in the non-homeostatic pathway - abnormalities akin to those observed in drug addicts - are likely to provide the neuroregulatory basis for compulsive appetite.

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Poster

695. Alcohol: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NSERC 311637

Title: Embryonic ethanol exposure affects numerical discrimination ability and sex preference of adult zebrafish (Danio rerio)

Authors: ***D. SEGUIN**[1], R. GERLAI[2];
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Abstract: The zebrafish is a highly social species which live in groups (shoals). Shoal living confers many advantages including protection from predation and increased access to mates and food sources. Past studies have found that early embryonic ethanol (EtOH) exposure in zebrafish leads to deficits in social responding. Based upon these past findings, the current studies have investigated the specificity of these altered social behaviours. Zebrafish embryos were exposed to one of three doses of ethanol (0.00%, 0.50% or 1.00% vol/vol EtOH) at 24h post fertilization for a period of 2 hours. Fish were tested during adulthood on numerical discrimination ability and sex preference. To test numerical discrimination ability, fish were placed in a test tank and exposed to two numerically different animated shoals presented simultaneously on computer monitors placed on opposite sides of the testing tank. Discrimination was measured by quantifying the distance to the numerically larger shoal. Shoal sizes of 4v0, 4v1, 6v2, 6v3, 8v2 and 8v4 were employed, and each experimental fish was tested once in each condition. Control (ethanol unexposed) zebrafish showed significant preference for the larger shoal especially in case of larger ratios. Sex preference was tested by placing zebrafish in a testing tank with a holding section on either side of the tank containing either an all-male shoal, an all-female shoal, or a mixed-sex shoal of live fish. Stimulus fish were separated by a clear, perforated Plexiglas wall, allowing the experimental fish to receive both visual and olfactory cues from the stimulus fish. Fish were tested once in each of the following conditions: all-male vs all-female, all-female vs mixed-sex and all-male vs mixed sex. Preference was measured by the total distance from each shoal the experimental fish swam, and the total amount of time spent near the stimuli shoals. Control female and male zebrafish were found to prefer all-female shoals over all-male and mixed-sex shoals. Results from zebrafish exposed to 0.5% or 1% (vol/vol) ethanol during their embryonic development showed significant impairment. Results will be discussed in the context of how to model cognitive alterations seen in Fetal Alcohol Spectrum Disorder children and in the context of how the employed zebrafish paradigms may be utilized in the analysis of underlying mechanisms.

Disclosures: **D. Seguin:** None. **R. Gerlai:** None.

Poster

696. Genetics of Addiction

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 696.01/N42

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant P50DA037844

Title: Individual differences in delay discounting by heterogeneous stock rats

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Abstract: Rats were tested on a sequential choice delay discounting (DD) procedure that simulates contingencies of reinforcement that are intended to be similar to those encountered by animals foraging in patchy environments. Thirsty rats chose between immediate small amounts of water and larger amounts of water that required the animal to travel to a new full patch. Long travel delays make staying in the old depleting patch a better choice, while short travel delays make long stays in the old depleting patch maladaptive. The sequential choice procedure differs from laboratory based "self-control" procedures typically used to study DD in non-human animals because repeated choice of the more immediate alternative can lead to greater consumption rates, whereas in "self-control" procedures choice of the delayed alternative always produces greater consumption due to experimenter imposed inter-trial intervals. It is arguable that the contingencies of reinforcement imposed by laboratory based "self-control" procedures are unlikely to be encountered in the natural environment. Optimal foraging theory predicts that animals will discount by delay to a degree that maximizes the overall rate of consumption. Here we tested a large number of heterogeneous stock rats (n=102) with highly variable genotypes on the sequential choice procedure with delays of 0, 6, 12, 18, 24 s. Rats were sorted according to how much they discounted by delay. Comparison of the twenty of rats that discounted the most (high discounters) with the 20 rats that discounted the least (low discounters) showed that the high DD yielded the greater water consumption rates. We also observed marked differences in switching between the two water feeders at the 0 s delay with some rats switching after every reinforcer while others stayed at the depleting water feeders for much longer periods before switching. Switchers and stayers were not different in the degree to which they discounted by delay. These results demonstrate that a propensity for DD can be advantageous by increasing overall consumption rate. Two behavioral phenotypes, high & low discounters and switchers & stayers were observed. Research is currently underway to identify genotypes that may underlie these patterns. This research may contribute to the identification of genetically determined biases in decision making underlying personality differences related to impulsivity.

Disclosures: J.B. Richards: None. **P.J. Meyer:** None. **S. Grisafi:** None. **G. Kelly:** None. **A. George:** None. **A.A. Palmer:** None.

Poster

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Program#/Poster#: 696.02/N43

Topic: C.17. Drugs of Abuse and Addiction

Support: P50DA037844

Title: Sex-dependent relationships between Pavlovian conditioned approach and cocaine-induced locomotion in heterogeneous stock rats

Authors: C. L. VERSAGGI[1], C. P. KING[1], *J. A. TRIPI[1], L. C. SOLBERG WOODS[2], A. A. PALMER[3], J. B. RICHARDS[4], P. J. MEYER[1];
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Abstract: Previous and ongoing research has sought to delineate complex genome-wide associations relating to traits of drug abuse. Heterogeneous stock (HS) rats allow us to investigate individual variability in these traits due to their robust genetic differences. In these experiments, we used two behavioral paradigms to investigate the relationship between these rats' responses to food- and cocaine-associated stimuli ("cues"), and whether this relationship depended on sex. Male (n=61) and female (n=60) heterogeneous stock rats were trained in a Pavlovian conditioned approach procedure, during which an 8-s illuminated lever predicted the delivery of a banana-flavored food pellet into a food cup. Rats received 25 trials daily for 5 days, and then were categorized as sign-trackers (ST; intense approach to the lever-cue), goal-trackers (GT; approach to the food cup), or intermediates (approach to both the cue and food-cup). Subsequently, rats were trained in a conditioned "cue" preference paradigm, during which they were injected with intraperitoneal saline or 10 mg/kg cocaine on alternating days before being placed in chambers with distinct textured floors. Cocaine- and saline-induced locomotion was measured using video tracking software. On the final day of testing, rats were injected with saline and placed in a chamber with both cocaine- and saline-paired floors. Time spent on the cocaine- vs. saline-paired floors was the primary measure of cocaine cue preference. We found no sex or ST/GT differences in preference for the cocaine paired floor. However, females showed more cocaine-induced locomotor activity than males. In addition, female sign-trackers had less cocaine-induced locomotion than female goal-trackers, and sign-tracking behavior was negatively correlated with cocaine-induced locomotion in females but not males. We conclude that the relationship between cocaine-induced locomotion and the responses to food- and cocaine-cues likely involve separable processes across sex. The long-term goal of the presented work is to determine how performance on these tasks can be influenced by genetic variants. As such, we predict that further work will elucidate the genotypic and phenotypic relationships between these tasks. Supported by P50DA037844

Disclosures: **C.L. Versaggi:** None. **C.P. King:** None. **J.A. Tripi:** None. **L.C. Solberg Woods:** None. **A.A. Palmer:** None. **J.B. Richards:** None. **P.J. Meyer:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: P50DA037844

Title: Sex-dependent correlations between addiction-related traits in heterogeneous stock rats

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Abstract: Drug addiction is associated with a number of traits related to behavioral regulation, including the response to novelty, attention, impulsivity, sensation seeking, and incentive salience attribution to cues. Understanding the relationship between these traits may be important for determining common behavioral and genetic processes involved in predisposition to addiction. As a first step in conducting a genome-wide association study of these traits, we tested 100 heterogeneous stock rats in six behavioral tests. We have initially focused on nine behavioral phenotypes, including locomotor activity (distance travelled), sensory reinforcement (responses that produce light onset), delay discounting (value of a delayed reinforcer), choice reaction time (reaction time and false alarms), Pavlovian conditioned approach (sign- and goal-tracking, and conditioned reinforcement), and cocaine-induced conditioned cue preference (preference for CS+, cocaine-induced locomotion). Initial analysis, indicates a subset of correlated measures, while many others appear to be unrelated. For example, sign-tracking and conditioned reinforcement were correlated, which suggests that these measures reflect partially overlapping neurobehavioral constructs. False alarms on the reaction time task were correlated with goal-tracking initially, but this correlation weakened as conditioning progressed. Sex differences were observed in several measures, with females showing more sign-tracking, sensory reinforcement, false-alarms, and conditioned reinforcement compared to males. Finally, several correlations among the measures were sex-specific. For example, locomotor activity was correlated with sensory reinforcement in males only, while it was correlated with indifference point only in

females. In conclusion, this initial analysis indicates that, while some measures are intercorrelated, most are not, and that in some cases the association appear to depend upon sex. Future studies will use genotype data from each rat to construct a genetic relationship matrix which will allow us to determine the extent to which any observed correlations are genetic rather than environmental in origin. We conclude that the genetic underpinning of these behaviors may be dissociable in a sex-dependent fashion. Supported by P50DA037844.

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Poster

696. Genetics of Addiction

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Burroughs Wellcome 9550300872

Title: Quantitative trait loci mapping of oxycodone reward and naloxone aversion in c57bl/6 substrains

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Abstract: Opioid addiction is a heritable substance abuse disorder, whose genetic basis remains poorly understood. Mammalian model organisms are valuable for identifying novel genes that contribute to variation in addiction-relevant intermediate phenotypes associated with various stages of addiction, such as opioid-induced psychomotor stimulation and reward. C57BL/6J (B6J) and C57BL/6NJ (B6NJ) strains show significant strain differences in several addiction-associated traits, including ethanol consumption, psychostimulant behaviors, and naloxone conditioned place aversion (Kirkpatrick and Bryant, 2014). Quantitative Trait Locus (QTL)

mapping in a Reduced Complexity Cross (RCC) between these substrains drastically reduces the number of genetic variants from millions (e.g., B6J vs. DBA/2J) to thousands, accelerating the rate at which the underlying quantitative trait genes (QTGs) can be identified. We conducted QTL mapping for oxycodone conditioned place preference (OXY-CPP; N=76), and naloxone conditioned place aversion (NAL-CPA; N=113), along with saline (SAL; N=83) controls. We utilized a 9 day CPP/CPA protocol; twenty four hours post-assessment of initial preference on Day 1 (D1), mice received drug (1.25 mg/kg OXY, 4 mg/kg NAL, or SAL) on D2 and D4 on the drug-paired side, and SAL on D3 and D5. Mice were assessed for drug-independent CPP/CPA (D8) and state-dependent CPP/CPA on D9 under the influence of drug. QTL mapping was performed in R/qtl using 96 informative markers (1000 permutations). Preliminary analyses identified two OXY-specific QTLs: D8-D1 mean visit on the drug-paired side (LOD=3.55; peak= Ch1 44.33 cM; $p<0.05$) and D9-D8 time on the OXY-paired side (LOD=3.49, peak=Ch11 42.15 cM, $p=0.05$). Additionally, we identified two overlapping NAL-specific QTLs: D9-D1 freezing bouts on the NAL-paired side (LOD=3.18; peak= ch18 31.67 cM; $p<0.10$) and D9-D1 time on the NAL-paired side (LOD= 2.98; peak= ch18 29.22 cM; $p=0.16$). We are currently increasing our sample size to N=150 per drug treatment and will use genome editing and transcriptome analysis to validate novel genetic and neurobiological factors underlying the motivational properties of opioids.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA033684

Title: Identifying human striatal gene networks and pathways associated with obesity

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Abstract: Drug addiction and obesity share common neurobiological mechanisms that regulate the excessive consumption of both addictive drugs and palatable foods. The neurologic influence of insulin and downstream signaling molecules provides a molecular link between feeding behaviors and reward in the mesolimbic and nigrostriatal dopamine (DA) system. We speculate that the obesity phenotype may be associated with dysregulation of coordinated gene networks of the ventral and dorsal striatum that process reward saliency. To test this hypothesis, we used RNA sequencing (RNA-seq) to identify genome-wide expression profiles in brain from non-obese and obese subjects. RNA-seq was used to survey the brain transcriptome of the caudate and nucleus accumbens from young subjects (n=25) over a range of body mass indices (BMI range, 18.7kg/m^2 - 40.7kg/m^2). RNA-seq alignment and splice junctions were determined from TopHat with resulting data fed to Cufflinks (v. 2.1) to assemble aligned RNA-seq reads into transcripts. In the caudate nucleus, we detected 185 transcripts that are associated with increasing BMI by linear regression at a false discovery rate of 0.5 (n=25). We identified 184 BMI-related transcripts by performing group comparison of normal weight (BMI $\leq$ 25 kg/m^2, n=7) and obese subjects (BMI $\geq$ 30 kg/m^2, n=9). Gene set enrichment analysis (GSEA) demonstrated dysregulated neurophysiological processes in the human caudate associated with neurotransmitter release, amine ligand binding receptors and lipid biology. Interestingly, the type 2-diabetes gene set was the top pathway that correlated with BMI (q-value=0.035, NES=-2.088). Preliminary quantification of insulin receptor (InsR) and phospho-Akt (Thr308) proteins in the dopamine nigrostriatal pathway demonstrates dysregulation of insulin-mediated signaling in brain. These findings suggest that overfeeding and increased body weight may be associated with central insulin resistance. Funded by PHS grant DA033684.

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Poster

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T32GM00A541

9550300872

Title: Hnrnph1 is a quantitative trait gene for methamphetamine sensitivity

Authors: *N. YAZDANI[1], C. C. PARKER[3], Y. SHEN[2], M. A. GUIDO[4], L. A. KOLE[4], S. L. KIRKPATRICK[1], J. E. LIM[4], G. SOKOLOFF[5], R. CHENG[6], W. JOHNSON[2], A. A. PALMER[4], C. D. BRYANT[1];
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Abstract: Sensitivity to the locomotor stimulant effects of amphetamines is a heritable trait in mice that may aid in our understanding of the genetic and neurobiological basis of neuropsychiatric disorders involving perturbations in dopaminergic transmission. We previously used short-term selected mouse lines derived from a C57BL/6J (B6) x DBA/2J (D2)-F2 cross to identify a quantitative trait locus on chromosome 11 that was causally associated with reduced methamphetamine-induced locomotor activity (D2 < B6). We replicated this QTL in a standard B6 x D2-F2 cross and used phenotypic analysis of interval specific congenic lines containing various D2-derived segments of chromosome 11 on an isogenic B6 background to uncover a 206 Kb critical interval containing only two protein-coding genes, Rufy1 and Hnrnph1, that was necessary for reduced MA sensitivity. Here, we used transcription activator-like effector nucleases (TALENs) to induce small deletions in the first coding exon of Rufy1 or Hnrnph1. Phenotypic analysis of replicate lines heterozygous for the Hnrnph1 deletion (Hnrnph1 hets) recapitulated the congenic phenotype while those heterozygous for the Rufy1 deletion did not, thus identifying Hnrnph1 as the quantitative trait gene. With regard to addiction-like phenotypes, Hnrnph1 hets displayed increased MA-induced conditioned place preference (MA-CPP) relative to WT B6 littermates at the 2 mg/kg dose. Transcriptome analysis via mRNA sequencing of B6.D2 congenic (chr.11: 50-60 Mb) striatal tissue followed by pathway analysis revealed perturbations in “glutamate receptor signaling” and “GalphaQ signaling”, and identified “Cellular development, nervous system development and function, behavior” as the top network. We hypothesize that Hnrnph1 regulates neurodevelopment of the mesocorticolimbic circuitry, thereby affecting both dopaminergic neuron development and glutamate signaling, and hence the stimulant response to amphetamines. These results will likely have widespread implications for understanding the genetic and neurobiological bases of disorders comprising perturbations in dopamine neurotransmission, including addiction, schizophrenia, ADHD, OCD, and Parkinson’s disease.

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Poster

696. Genetics of Addiction

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Title: A polymorphism in the OPRM1 3' untranslated region is associated with methadone efficacy in treating opioid dependence

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Abstract: There is a wide range of outcomes for opioid dependent patients treated with either methadone or Suboxone. A significant percentage of patients do not have successful treatment outcomes and continue to use opioids other than the ones prescribed. Both environmental factors and genetic variation contribute to these differences in treatment efficacy in the patient population. Identification of relevant genetic markers may allow individuals to be prospectively genotyped and then prescribed the medication with the best chance of working. *OPRM1* encodes the mu-opioid receptor (MOR), the primary receptor for opioids of abuse, and single nucleotide polymorphisms (SNPs) in the gene may be important to the pharmacogenetics of opioid addiction treatment. The primary *OPRM1* transcript in neurons is MOR-1, which has a large ~11kb 3' untranslated region (UTR). The MOR-1 3' UTR has five common haplotypes (>10% frequency) in European-Americans and these haplotypes are tagged by 5 SNPs. Since 3' UTRs are often involved in regulating gene expression, we analyzed these 5 SNPs in 566 European-American patients enrolled in the Starting Treatment with Agonist Replacement Therapy (START) clinical trial. The START trial was a 24 week, open-label trial, which randomized opioid dependent patients to either methadone or Suboxone. One *OPRM1* SNP, rs10485058, was

significantly associated with the percentage of weekly opioid positive urine drug screens in patients prescribed methadone ($p = 0.002$). Individuals with the A/A genotype had 33.4% positive urines over the 24 weeks of the trial, while those with either the A/G or G/G genotypes had 48.6% positive urines. No effect was present in the Suboxone population. When analyzed using a generalized estimating equation to account for the effects of time, sex, and age, rs10485058 was again significant in the methadone population ($p = 0.007$). To further study this finding, rs10485048 was analyzed in an independent Australian cohort of opioid addicts undergoing agonist replacement therapy ($n = 1215$). Patients with the A/A genotype were more likely to self-report abstinence during the trial compared to individuals with the A/G or G/G genotypes, supporting the initial association. *In silico* analysis of microRNA binding sites near rs10485058 revealed a miR-95-3p site predicted to be disrupted by the A allele of the SNP, providing a potential mechanism of action for the observed pharmacogenetic effect. We hypothesize that individuals carrying the G allele of rs10485058 express lower levels of MOR-1 protein than those with the A/A genotype, reducing the ability of methadone to prevent illicit opioid use.

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Poster

696. Genetics of Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: Indian Council of Medical Research, New Delhi, INDIA

Title: Association of GABRA2 polymorphisms and haplotype analysis in Alcohol Dependence

Authors: *B. M. SHANKARAPPA;
psychiatry, Natl. Inst. of Mental Hlth. and Neuroscien, Bangalore, India

Abstract: Aim: 1. To analyze the effects of polymorphisms (rs279836, rs279845 and rs279871) in the GABRA2 gene on the risk of alcoholism. 2. To determine associations between genetic markers basing on haplotype analysis. Background: Alcohol consumption, particularly heavier drinking, is an important risk factor for many health problems and, thus, is a major contributor to the global burden of disease. The major inhibitory neurotransmitter γ-aminobutyric acid (GABA) has been studied extensively in the human CNS. Genetic variation in GABA may be an important source of risk for developing alcohol dependence based on evidence. Evidence suggests that alcohol initially potentiates GABA effects; increases inhibition, and often the brain becomes mildly sedated. But over time, chronic alcohol consumption reduces the number of GABA receptors through the process of down-regulation. When alcohol is eventually withdrawn, the loss of its inhibitory effects, combined with a deficiency of GABA receptors, may contribute to over-excitation through-out the brain. This effect, in turn, can contribute to withdrawal seizures. Methods: In the present study we have analyzed the GABRA2 SNPs among AD cases (N=200) and age matched controls (N=124). Male participants were selected from patients seen at the Centre for Addiction Medicine, NIMHANS. Individuals who met the criteria for alcohol dependence (ICD 10) were recruited into the study after obtaining informed consent. The clinical instruments applied included Semi Structured Assessment for Genetics of Alcoholism-IV, Severity of Alcohol Dependence score, MINI psychiatric interview, scale for family interview and Revised combined clinical & laboratory index for alcoholic liver disease. DNA isolated from the peripheral blood was used for genotyping, done by Polymerase Chain Reaction followed by Restriction Fragment Length polymorphism method for rs279845 and by predesigned TaqMan® SNP genotyping assays in RT-PCR for rs279836 and rs279871 was performed. Haplotype analysis is performed using UNPHASED software. Results: Among the three SNPs analyzed none of them showed significant difference between AD subjects and controls. All three SNPs were in LD (r2= 0.99) and they are in strong association when present together in haplotype , which is significant in cases but not in controls (Likelihood ratio chisq = 29.285 df = 5 p-value = 2.03857e-005) Conclusion: Even though there is a absence of single SNP association with alcoholism. The Haplotype analysis showed there is a strong association of these 3SNPs with alcoholism

Disclosures: B.M. Shankarappa: None.

Poster

696. Genetics of Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH IRP (NIDA)

NIH HD25938

Title: Reduced cadherin 13 expression alters dopaminergic fibers and cocaine-related behaviors in mice

Authors: *G. R. UHL[1], J. DRGONOVA[2], L. HARTSTEIN[2], M. BAUMANN[3], B. RANSCHT[4]; [1]Mol. Neurobio., NMVAHCS, BRINM and NIH/NIDA, Albuquerque, NM; [2]Mol. Neurobio., [3]Medication DIscovery, NIH/NIDA, Baltimore, MD; [4]Sanford-Burnham Reserach Inst., LaJolla, CA

Abstract: Proper function of brain circuitry requires accurate expression and regulation of cell adhesion molecules (CAMs), including those of the calcium-dependent cadherin family. Cadherin-13 (*CHD1*3; T-cadherin) is an atypical cadherin in that it lacks an intracellular domain and is localized to the plasma membrane *via* a glycosylphosphatidylinositol anchor. Molecular genetic studies in humans associate variants in *CDH13* with vulnerability to substance dependence, reward from modest amphetamine doses and ability to quit smoking. In mice, Cdh13 mRNA is expressed in cerebral cortex, hippocampus, amygdala, striatum, ventral tegmental area, and other brain regions implicated in addiction circuitry. *CDH13* knockout mice (*CDH13-KO*) provide a good model for possible behavioral and anatomical influences of variation at this gene locus. *CDH13-KO* mice exhibit increased sensitivity to cocaine reward as assessed by conditioned place preference test, and increased densities of dopaminergic fibers in cingulate cortex, consistent with *in vitro* observations that homophilic CDH13 interactions inhibit neuronal growth. The changes in dopaminergic innervation are accompanied by neurochemical evidence for increased dopamine turnover in the cortex but not in other brain areas tested. These behavioral, neurochemical and anatomical observations are consistent with contributions of variants in this gene to human addiction phenotypes. *Support: NIH IRP (NIDA); NIH HD25938 (BR).*

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Poster

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MJGF is a 'Ramon y Cajal' Researcher (MINECO, Spain)

Title: Adolescent cocaine experience differentially augments adult sensitization and alters nucleus accumbens epigenetic profiles in selectively bred rats that differ in addiction liability

Authors: *A. PARSEGIAN[1], J. GARCIA-FUSTER[2], P. BLANDINO[1], S. J. WATSON, Jr[1,3], S. B. FLAGEL[1,3], H. AKIL[1,3];
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Abstract: While many people experiment with drugs of abuse, only a small minority go on to become drug addicts. Genetic predisposition is implicated, but early initiation of drug use in adolescence also reliably predicts the likelihood of addiction in adulthood. Thus, adolescence may be a critical period during which drug use can elicit neuroadaptations that might render one more susceptible to addiction in adulthood. There has been growing evidence for an important relationship between chromatin modifications, the genes they modify, and the enduring neurobiological impact of cocaine in promoting addiction. Using a unique genetic rat model that captures individual differences in vulnerability to addiction, we asked whether adolescent drug exposure alters their genetic predisposition via epigenetic chromatin modification. Rats selectively bred based on locomotor response to novelty also differ on a number of addiction-related traits. Specifically, relative to bred low-responder (bLR) rats, bred high-responders (bHR) are more sensitive to the psychomotor-activating effects of cocaine and reinstate drug-seeking more readily following a prolonged period of abstinence. We exposed these bred rats to a sensitizing regimen of cocaine (15 mg/kg) or saline for 7 days during adolescence (PND 33-39) and assessed phenotypic differences in the acute and sensitized response to cocaine during this

period. We also tested some rats, with or without a history of adolescent cocaine exposure, for response to cocaine in adulthood (PND 77-84). We then measured two histone modifications, acetylation (ac) and tri-methylation (me3) on histone 3 lysine 9 (H3K9) in the nucleus accumbens (NAc) core and shell during either adolescence, or adulthood. As expected, bHRs showed greater sensitization than bLRs to repeated cocaine exposure during both adolescence and adulthood. However, adolescent cocaine exposure shifted the bLR phenotype to express greater sensitization in adulthood. Conversely, bHRs with prior adolescent cocaine showed blunted expression of sensitization in adulthood. Adolescent cocaine also induced epigenetic differences in bHRs relative to saline controls, with an increase in both the repressive mark H3K9me3 in the shell and the permissive mark acH3K9 in the core. By comparison, in bLRs, adolescent cocaine reduced both marks in the core, but had no significant change in the shell, relative to their saline controls. Importantly, all of these epigenetic changes were present in adolescence and persisted into adulthood. These results indicate that adolescent drug use might influence inborn genetic addiction liability via epigenetics.

Disclosures: **A. Parsegian:** None. **J. Garcia-Fuster:** None. **P. Blandino:** None. **S.J. Watson:** None. **S.B. Flagel:** None. **H. Akil:** None.

Poster

696. Genetics of Addiction

Location: Hall A

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Topic: C.17. Drugs of Abuse and Addiction

Support: KISTIF Funding 2E24480

WISET Grant 2N38873

Title: Serum exosomal microRNA-137 is a potential biomarker for repeated psychostimulant exposure

Authors: ***H.-I. IM**, E. NAM, B. KIM, J. KIM, S. LEE, H. KIM, J. WOO;
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Abstract: Current biological assay for detecting psychostimulant exposure is applicable up to few months of abstinence, but determining psychostimulant exposure after several months to years of abstinence and distinguishing acute/repeated psychostimulant exposure are still difficult. A significant amount of exosomal microRNAs (miRNAs) are circulating in remarkably stable

forms within blood serum, and some miRNAs have a high degree of brain specificity. Thus the brain-originated exosomal miRNAs in serum may prove to be suitable for detecting long-term changes in brain caused by repeated psychostimulant exposure. Here we demonstrate on complementary studies using mouse brain tissue and blood, patient blood samples, biochemical assays and transgenic approaches to show that both striatal and serum exosomal miR-137 are concordantly diminished in mice after repeated cocaine exposure, and that the reduction in serum exosomal miR-137 can effectively distinguish the individuals with history of chronic methamphetamine addiction. Additionally, we provide evidences that striatal and serum exosomal miR-137 levels correlate with each other after repeated cocaine exposure, and that striatal biomolecules could be directly delivered by blood-to-brain transport. Overall, our data suggest that the exosomal miR-137 in peripheral bloodstream can potentially serve as a non-invasive, sensitive and reliable biomarker for repeated psychostimulant exposure.

Disclosures: **H. Im:** None. **E. Nam:** None. **B. Kim:** None. **J. Kim:** None. **S. Lee:** None. **H. Kim:** None. **J. Woo:** None.

Poster

696. Genetics of Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NSFC of China 81471350

SF of Ningbo,China 2013A610252

Title: Significant association of rs2240158 in the glutamate receptor subunit gene (GRIN3B) with heroin addiction

Authors: ***X. XIE**[1,2,3], H. LIU[1,2,3], W. ZHOU[1,2,3];
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Abstract: Objective Growing evidence suggest that N-methyl-D-aspartate (NMDA) receptor-mediated glutamate neurotransmission may be involved in the pathophysiology of drug addiction. The NMDA receptor is consisting of three subfamilies (NR1, NR2 and NR3). The ability of NR3 to negatively modulate the NMDA receptor function makes it an attractive

candidate gene of heroin addiction. The purpose of this study is to explore the association between two single nucleotide polymorphisms (SNPs) (rs4807399, rs2240158) in the glutamate receptor subunit gene (GRIN3B) and heroin addiction. Methods The genotypes of the two SNPs (rs4807399, rs2240158) in heroin dependent patients and normal control subjects of the male Han Chinese were detected by TaqMan SNP genotyping method, and the association between heroin dependence and the two SNPs were analyzed. Results The frequencies of genotype and allele at rs2240158 were significantly different between the cases and the controls (nominal P were 0.0168, 0.0062, respectively). The distributions of genotype and allele at rs4807399 were not significantly different between in the cases and in the control group ($P > 0.05$). In addition, the C allele frequency of rs2240158 was significantly higher in cases compared with the control group (OR = 1.603, 95% CI 1.142-2.252, P = 0.0062). And the frequency of CC genotype of rs2240158 was significantly higher in cases compared with the control group (OR = 1.599, 95% CI 1.086-2.354, P = 0.0171). Conclusion Our study indicates that the rs2240158 of the glutamate receptor subunit gene (GRIN3B) play a major role in heroin addiction.

Disclosures: **X. Xie:** None. **H. Liu:** None. **W. Zhou:** None.

Poster

696. Genetics of Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant# U54 HG006332

Title: Identification of addiction-relevent genes using high-throughput drug naïve behavioral screens in the knock out mouse project (KOMP)

Authors: *P. E. DICKSON[1], T. WILCOX[1], J. NDUKUM[1], J. CLARK[1], J. A. BUBIER[1], S. J. RIZZO[1], J. C. CRABBE[2], J. M. DENEGRE[1], K. L. SVENSON[1], R. E. BRAUN[1], V. KUMAR[1], E. J. CHESLER[1];
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Abstract: Given the substantial evidence that predisposing behaviors predict subsequent addiction and the prevalence of dual diagnosis of addiction, alcoholism and other disorders, we expect that these behaviors will predict the tendency to self- administer drugs, and therefore enable identification of clinically relevant addiction genes using drug-naïve mice. Behavioral phenotypes were incorporated into The Jackson Laboratory Knockout Mouse Phenotyping

(KOMP) pipeline based on predicted relevance to addiction and mood disorders. To test the predictive validity of these assays for alcohol and drug related phenotypes, strains that exhibited significantly different phenotypes relative to C57BL/6NJ controls on at least one behavioral assay with relevance to anxiety, depression, or response to novelty are being tested on three different addiction-relevant assays: nicotine two bottle choice, methamphetamine two bottle choice, or ethanol two bottle choice. We predicted that anxiety, reactivity and depression mutants will have increased alcohol consumption, anxiety and depression mutants will have increased nicotine consumption, and novelty response/exploratory behavior mutants will have increased methamphetamine consumption. Results support this predictive relationship. For example, Htr3b knockout mice are anxiety/reactivity mutants that also show significant increase in ethanol drinking in the two bottle choice test. These studies provide an indication of the predictive value of the additional behaviors in the JAX KOMP pipeline for addiction research and will inform decisions about future inclusion of related traits.

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Poster

697. Cross-Modal Processing: Spatial Factors

Location: Hall A

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Topic: D.03. Multisensory Systems

Support: DFG Grant 01EO1401

Title: Tolerance for perceiving a stable world depends on visual and vestibular variability

Authors: *I. GARZORZ[1], P. R. MACNEILAGE[2];
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Abstract: Whenever the brain encounters simultaneous signals from different sensory modalities, it has to judge whether these signals originate from the same external event or object. This process implies comparison of sensory signals leading to integration if signals agree or segregation if they don't. While statistically optimal cue integration has been widely studied, there is less work on probabilistic signal comparison. A straightforward model for sensory cue comparison implies subtraction of sensory signals leading to a difference distribution with mean

equal to the difference of compared signal means, and variance equal to the sum of compared signals variances. When this distribution has mean significantly different from zero, signals are likely to have independent origin. To test this model for visual-vestibular cue comparison we conducted an experiment using a virtual reality set-up allowing for independent control of visual and vestibular stimulation. The stereo visual scene consisted of red spheres (diameter = 0.6 cm; density = 0.004 spheres/cm^3; clipping planes: near = 25 cm, far = 65 cm) and a head-fixed fixation point. Lateral translations had a Gaussian velocity profile of constant duration (0.8 s) and displacement of 5 cm for the reference movement (peak velocity = 0.125 m/s). The variances associated with visual and vestibular estimation of self-motion were measured in two single-cue amplitude discrimination conditions using a 2IFC task and an adaptive staircase procedure. These unimodal measurements of eight participants were used to make subject-specific predictions about response variability in a third condition with simultaneous visual and vestibular stimulation and a visual gain factor that was varied from trial to trail. On each trial, subjects had to indicate whether the visual scene moved with or against them in world coordinates, which required an evaluation of visually-simulated self-motion speed relative to perceived self-motion speed. The visual gain factor that elicited an equal number of "with" versus "against" responses leads to perception of a stable visual environment. Previous studies have reported gains greater than one, but we observed gains less than or equal to one. Previous studies have not explicitly related visual gain variability to single-cue variability measurements. Supporting the difference-distribution model, we observed that variance on visual-gain judgments, which quantifies tolerance for discordance in perceiving a stable visual world, is well-predicted by the sum of measured variances on single-cue estimates.

Disclosures: I. Garzorz: None. **P.R. MacNeilage:** None.

Poster

697. Cross-Modal Processing: Spatial Factors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 697.02/O8

Topic: D.03. Multisensory Systems

Support: FP7-ICT 611452

Title: Audio-motor spatial integration during curvature exploration

Authors: *S. FINOCCHIETTI[1], G. CAPPAGLI[1], E. COCCHI[2], M. GORI[1];
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Abstract: ABBI, the Audio Bracelet for Blind Interaction, is a bracelet that is worn on the wrist and provides an audio feedback about body movements to help visually impaired children to build a sense of space and facilitate the social interactions. This study was designed to assess the effectiveness of ABBI system for improving mobility and spatial cognition in visually impaired children. Eight low vision children (aged 9-16, with residual vision lower than 1/10) took part in this study. The study lasted 12 weeks. Once per week each child participated in a 45-minutes spatial rehabilitation session where he was blindfolded and performed different mobility and spatial cognition exercises. He also had to use it one hour per day at home alone or with one relative. The mobility and spatial cognition abilities were measured before and after the 12-weeks rehabilitation program with three different tests targeting the proprioceptive, audio and motor abilities. Results showed that the use of the Audio Bracelet for Blind Interaction allowed the low vision child to improve their mobility and audio abilities.

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Poster

697. Cross-Modal Processing: Spatial Factors

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Program#/Poster#: 697.03/O9

Topic: D.03. Multisensory Systems

Title: Audiovisual integration in areas MT & MST of marmoset monkeys

Authors: *T. A. CHAPLIN[1,2,3], B. J. ALLITT[1], M. A. HAGAN[1], N. S. C. PRICE[1,3], R. RAJAN[1,2], M. G. P. ROSA[1,2,3], L. L. LUI[1,3];
[1]Physiol., [2]ARC Ctr. of Excellence for Integrative Brain Function, [3]Monash Vision Group, Monash Univ., Melbourne, Australia

Abstract: The traditional model of sensory processing states that each modality is processed independently and is only integrated in higher-level multisensory cortical areas. However, recent studies have shown anatomical and physiological evidence for multisensory integration in regions that have historically been attributed to one sense. The middle temporal area (MT) and the medial superior temporal area (MST) of the primate cerebral cortex have well known roles in processing visual motion, but in marmosets they are known to have direct connections with auditory cortex (although these connections are more abundant for MST than MT). Therefore we tested if neurons in these areas are responsive to auditory motion stimuli, and whether these neurons can integrate auditory and visual motion cues. We performed extracellular recordings (n = 29, 24 in MT, 5 in MST) in 3 anaesthetised marmosets and measured the neurometric

thresholds of neurons for visual, auditory, and audiovisual motion stimuli. Visual stimuli were random dot kinematograms moving either left or right. Auditory stimuli were interaural level difference ramps of 6-12kHz bandpass noise presented with headphones which simulated auditory motion. The strength of the visual motion signal was manipulated by decreasing the coherence of the dots, whilst the auditory motion signal was weakened by adding background white noise in both ears. Auditory and visual stimuli were presented at the same spatial location, direction and speed for 1 second with matching noise levels, to maximise the chance of neurons showing multisensory integration and enhancing neurometric thresholds in the audiovisual conditions. None of the neurons from either area was reliably responsive to auditory stimuli alone. While there were some circumstances where auditory stimuli appeared to modulate visual responses in MT, auditory stimuli which complemented visual motion did not reliably improve neurometric thresholds in any of our MT cells in a left-right discrimination paradigm. Only one neuron from area MST showed a statistically significant change in threshold between the visual and audio-visual conditions. These data, while not ruling out minor modulatory influences from the auditory domain, suggest that MT does not integrate auditory cues for motion. It is more likely this occurs in downstream areas such as MST, VIP or beyond.

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Poster

697. Cross-Modal Processing: Spatial Factors

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Topic: D.03. Multisensory Systems

Title: The relationship between body representation and visuospatial perception

Authors: ***K. NAKATA**, S. NOBUSAKO, S. MORIOKA;
KIO UNIVERSITY, Nara, Japan

Abstract: [Objective] Illusory perceptions of rubber hand ownership that are induced by the rubber hand illusion (RHI) modulate pseudoneglect. That study reported that the subjective midpoint of a line in a bisection task shifts from left to right in response to the left-handed RHI. However, the cause of this shift is unclear. One possibility is that the motor response changes when the line is bisected. This study was conducted to determine if the left-handed RHI affected the gaze behavior of the premotor response in the line bisection task. [Method]The participants were 23 healthy right-handed adults (12 males, 11 females, mean age: 25.0, standard deviation:

3.3). RHI was done with the left hand to change body representation. The rubber hand was placed 20 cm to the right of the actual left hand. Brush stimulation was performed for 3 min in synchronous and asynchronous conditions. The illusion was evaluated with proprioceptive drift and questionnaires. The line bisection task was conducted with 20-cm and 30-cm line segments before and after the RHI and evaluated with an eye tracker (Tobii Studio). The participants were asked to watch the subjective midpoint of each line for 10 s. The area of interest was set from the center of each line segment to the left and right, and the total fixation duration (s) and gaze points (pixels) were compared before and after the RHI. [Results]Proprioceptive drift was larger in synchronous conditions than in asynchronous conditions. Questionnaire items that reflected the sense of ownership and agency, location, and loss of own hand were significantly higher ($p < 0.05$) in synchronous conditions than in asynchronous conditions. Deviation of the gaze point on the left side of the actual midpoint of the 20-cm line was observed before the RHI, and the gaze point shifted significantly to the right after the illusion ($p < 0.05$). The changes in the gaze points and the proprioceptive drift were positively correlated ($r = 0.6000$, $p = 0.0181$). The total fixation duration in the area of interest before the RHI was longer on the left side, and the illusion later showed a tendency to increase the total fixation duration on the right side. [Conclusion]A series of behavioral changes shifted the gaze point to the right due to the right-sided RHI, which has been suggested to be due to the perception of a subjective midpoint of a line segment by a change of body representation. In addition, the questionnaires showed that the consciousness structure of embodiment(sense of ownership and agency, location, and loss of own hand) was an important factor in visuospatial perception. However, these changes were behavioral, and the underlying mechanisms were not clear.

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Poster

697. Cross-Modal Processing: Spatial Factors

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Topic: D.03. Multisensory Systems

Support: ERC-2009-AdG 249425-CriticalBrainChanges

Title: Tactile and crossmodal localization in adults with autism spectrum disorder

Authors: ***M. HENSE**[1], S. BADDE[1], S. KÖHNE[2], J. HABICH[1], I. DZIOBEK[2], B. RÖDER[1]; [1]Univ. of Hamburg, Hamburg, Germany; [2]Humboldt Univ. of Berlin, Berlin, Germany

Abstract: Skin-based, anatomically anchored coordinates of a touch seem to be automatically remapped into external space by taking into account body posture; the final location estimate likely results from a weighted integration of information from multiple reference frames. Remapping of tactile stimuli is a prerequisite for multisensory integration of tactile and visual stimuli, which are initially coded in different, modality-specific reference frames. Prospective studies in children have suggested that multisensory localization follows a protracted developmental time course reaching adult level in early adolescence. There is evidence that children with Autism Spectrum Disorder (ASD) may stronger weight anatomical reference frames when localizing tactile stimuli. However, it might be argued that this finding arose from a developmental delay and would thus have disappeared by reaching adulthood. Finally, possible consequences of altered touch localization for multisensory processing is not yet known. In the present study, 16 high-functioning adults with ASD and 16 typically developed controls performed both a tactile temporal order judgment task (TOJ-task) as well as a visuo-tactile crossmodal congruency task (CC-task) while adapting either an uncrossed or crossed hand posture. In both tasks participants had to localize a tactile target stimulus at one hand in the presence of either a tactile (TOJ-task) or a visual (CC-task) distractor stimulus, respectively. Since with crossed hands anatomical and external coordinates are in conflict, a comparison of touch localization between both postures allows estimating the weights assigned to the anatomical vs. external reference frame. Results for both tasks were indistinguishable between groups. Therefore, we concluded that the automatic remapping of touch into external space and the weighting of reference frames is not affected by ASD provided that developmental delays are taken into account.

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Poster

697. Cross-Modal Processing: Spatial Factors

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PIFI-PROMEP-VIEP

Catedra-Moshinsky

VIEP-BUAP MEBI-EDH-15

Title: Mechanisms of multisensory stochastic resonance in the superior colliculus

Authors: *N. HUIDOBRO[1], I. MENDEZ-BALBUENA[2], B. DE LA TORRE VALDOVINOS[1], E. MANJARREZ[1];
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[2]Maestría en Diagnóstico y Rehabilitacion Neuropsicológica, Benemerita Univ. Autonoma de Puebla, Puebla, Mexico

Abstract: In previous studies we described the occurrence of a phenomenon named multisensory stochastic resonance (SR), which is exhibited in psychophysical experiments in humans (Manjarrez et al., 2007) and electrophysiological experiments in the superior colliculus in cats (Huidobro and Manjarrez, 2014; Abs Soc Neurosci 331.03). Here we extended these studies by means of single unit recordings in several regions of the superior colliculus which are conceivable involved in multisensory integration. Experiments were performed in three precollicular-post mammilary decerebrate cats. Extracellular unitary recordings were obtained by means of quartz-platinum/tungsten microelectrodes from Thomas Recording (5 to 7 MΩ). We recorded 215 neurons distributed in the stratum zonale (45), stratum griseum superficiale (59) and stratum griseum intermediale (111). The input signal consisted of periodic-subthreshold visual stimuli elicited by a pair of LEDs. In addition, we applied continuous auditory noise with a pair of headphones adapted to the cat eardrum. We found that most of the neurons did not exhibit a background activity and they were in subthreshold conditions until an optimum level of auditory noise induced its firing. Furthermore, the total number of spikes measured from the peristimulus histogram exhibited an inverted U-like form as a function of the auditory noise level. We did not observe statistically significant differences in the strength of the SR at optimum noise among the different layers of the superior colliculus. We suggest that the multisensory SR observed in the superior colliculus, under visual stimulation, is mediated by the activation of subthreshold cross-modal neurons which reach the threshold at an optimal level of auditory noise.

Disclosures: N. Huidobro: None. **I. Mendez-Balbuena:** None. **B. de la Torre Valdovinos:** None. **E. Manjarrez:** None.

Poster

697. Cross-Modal Processing: Spatial Factors

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BBSRC CIP BB/J009849/1

Wellcome Trust TDG WT091681MA

Title: Spatial influences on audio-visual interactions in the monkey brain

Authors: *R. S. MUERS[1], M. J. BARTOLO[1], T. D. GRIFFITHS[1,2], A. THIELE[1], C. I. PETKOV[1];
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Abstract: Multisensory signals that are in spatial and temporal register modulate a number of cortical areas. Many such regions, including those in caudal auditory cortex, belong to a dorsal, presumably spatial-processing pathway. However, brain imaging studies in nonhuman animals have not directly assessed the impact of spatial influences on audio-visual interactions. Thereby, an epistemic gap exists between human neuroimaging and nonhuman animal electrophysiological studies on spatial multisensory interactions. To advance our understanding, we used spatially moving audio-visual stimuli during functional MRI with two Rhesus macaques. Tonotopic maps for each animal were also obtained to approximate the location of auditory cortical fields. The monkeys were presented with unimodal (auditory or visual) and bimodal (audio-visual) stimuli moving in azimuth. Auditory stimuli were, virtual-acoustic space, moving broadband noises. Visual stimuli were moving black squares on a gray background. Bimodal stimuli either moved congruently or incongruently in spatial relation to each other. We analysed the fMRI data seeking to identify brain regions showing supra-additive multisensory interactions (modelled as: Bimodal (AV) > Unimodal (A+V)). Significant supra-additive interactions were observed in a number of brain areas (cluster corrected, $P < 0.01$) including areas VIP and LIP in the intra-parietal sulcus, area TPO in the superior-temporal sulcus, and visual area MT. Primary auditory and visual cortical areas also showed strong multisensory interactions. Stronger multisensory interactions were observed in posterior auditory cortical fields, including field A1 (RM-ANOVA, $P < 0.01$) and incongruent audio-visual stimuli preferentially modulated postero-medial auditory cortex. The multisensory effects in auditory cortex showed some interesting differences between the left and right hemispheres, although at the whole-brain level the effects were largely bilaterally distributed. In a control experiment with one of the macaques, we evaluated whether interactions were specific to moving stimuli. We observed that moving unimodal stimuli were more effective than stationary stimuli in activating caudal auditory fields and visual area MT. However, moving and stationary supra-additive multisensory interactions were comparable in these regions. Our results identify spatial

influences on audio-visual interactions in the primate brain and serve as a bridge between human neuroimaging studies and those in animal models studying multisensory interactions at the neuronal level.

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Poster

697. Cross-Modal Processing: Spatial Factors

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Support: Wellcome Trust Grant WT07650AIA

Title: Neural correlates of multisensory behavior in the auditory cortex

Authors: A. HAMMOND-KENNY[1], V. M. BAJO[1], A. J. KING[1], *F. R. NODAL[2];
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Abstract: Our perception of everyday events relies on the ability to integrate information obtained from different sensory modalities. Such integration often results in shorter detection times and greater accuracy or discriminability. Recent neural recording studies have shown that multisensory processing occurs even at the level of early sensory cortices. However, to date, these studies have mainly been performed under anesthesia and, therefore, the behavioral relevance of the cross-modal interactions observed remains to be determined. Here, we explore the multisensory nature of the ferret auditory cortex by recording neural activity while animals perform different tasks, thereby enabling correlation of neural and behavioral changes. Ferrets were trained by positive operant conditioning in two audio-visual tasks. Task 1 tested the performance accuracy of animals when localizing multisensory stimuli as compared to component unisensory stimuli, presented from 1 of 7 sites separated by 30° around the frontal hemifield of a circular arena. Task 2 tested the ability of ferrets to categorize multisensory stimuli according to the spatial congruency of their unisensory components. Once behavioural abilities were established and performance was stable, neural activity was recorded during task performance via bilaterally implanted electrode arrays. Behaviorally, animals showed an improvement in localization accuracy for multisensory versus component unisensory stimuli, with greatest gains observed at the shorter stimulus durations (<200 ms) and at more lateral locations. In addition, Race model inequality analysis of approach-to-target response times and

head orienting reaction times showed that different mechanisms, multisensory integration and probability summation, respectively, were responsible for the multisensory facilitation observed in the two response modes. Recordings from cortical neurons showed that approximately 40% of units responded to both stimulus modalities, with the majority (>90%) of bisensory units displaying sub-additive interactions to spatially congruent audio-visual stimuli presentations. In addition, although ferrets can correctly identify spatially congruent multisensory stimuli, bisensory units exhibited higher firing rates to spatially incongruent than to congruent stimuli presentations. Together, our results suggest that multisensory activity is widespread in the auditory cortex and that the magnitude of the interactions between visual and auditory inputs changes when the stimuli are categorized behaviorally according to their relative locations.

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Poster

697. Cross-Modal Processing: Spatial Factors

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Topic: D.03. Multisensory Systems

Support: DFG HE 6368/1-1

Title: Assigning a touch on the hand to a foot: post hoc construction of tactile location

Authors: *T. HEED[1,2], B. RÖDER[2], S. BADDE[2];
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Abstract: Touch activates primary somatosensory cortex (S1), and tactile skin location is reflected in the homuncular organization of this cortical region. However, human participants sometimes confuse tactile locations, for example, when reporting which of two stimuli, one on each hand, occurred first while the hands are crossed [1, 2]. This finding is thought to indicate that tactile location is estimated by integrating skin-based and spatial information, as skin and space indicate opposite sides with crossed hands [3]. Yet, though caused by spatial conflict, such confusions may index errors in choosing between the two stimuli rather than spatial mislocalization of the first stimulus to the location of the second. Here, we show that participants systematically mislocalize touch to body parts that have not been stimulated, and, accordingly, whose associated S1 region has not received peripheral input. Participants sat on the floor, resting their hands on a see-through platform just above their outstretched feet. To dissociate

body side from side of space, hands and feet could each take on uncrossed and crossed postures. In each trial, two of the four limbs were tactually stimulated in short succession. Participants reported which limb had received the first touch, either by moving the respective limb, or by calling out the color of a sleeve worn on it. Independent of response mode, participants made two kinds of errors. First, they often reported the second touch to have occurred first, and more so when the limb receiving the first touch was crossed than when it was uncrossed (15 vs. 20%). Second, they often reported touch on a limb that had not been stimulated at all (10%). Both error types systematically reflected three characteristics: Participants erroneously reported the homologous limb (e.g., left hand response for right hand stimulus); they reported the limb of the same body side (e.g., left foot response for left hand stimulus); and they reported limbs on the same side of space (e.g., response with crossed left foot for stimulus on uncrossed right hand). In a new experiment, participants received only a single touch per trial. Again, they reported touch on non-stimulated limbs, with fewer, but similar, systematic errors as for the two-stimulus paradigm. In sum, tactile localization to a limb can occur without any peripheral tactile input to the respective S1 region, resulting in drastic mislocalization, for example across hands and feet. Accordingly, our results imply that tactile location is constructed post hoc by integrating skin location with three types of body-related and spatial information about the touched limb: limb type (hand vs. foot), body side, and side of space.

Disclosures: **T. Heed:** None. **B. Röder:** None. **S. Badde:** None.

Poster

697. Cross-Modal Processing: Spatial Factors

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.03. Multisensory Systems

Support: Deutsche Forschungsgemeinschaft GK 1247/2

Deutsche Forschungsgemeinschaft SFB-TR 31/TPA8

Title: The role of auditory cortex in the audiovisual ventriloquist aftereffect

Authors: *B. ZIERUL[1], B. RÖDER[1], C. TEMPELMANN[2], P. BRUNS[1], T. NOESSELT[3,4]; [1]Biol. Psychology and Neuropsychology, Hamburg Univ., Hamburg, Germany; [2]Dept. of Neurol., [3]Inst. of Biol. Psychology, [4]Ctr. for Behavioral Brain Sci., Otto-von-Guericke Univ., Magdeburg, Germany

Abstract: When presented alongside a simultaneous but spatially incongruent visual stimulus, localization of an auditory stimulus is usually biased toward the location of the visual stimulus. This is known as the ventriloquist effect (VE). Exposure to a train of audiovisual stimuli, where the visual stimuli are displaced from the auditory stimuli in a constant direction, can bias later localization of unisensory auditory stimuli in the direction of displacement, which is known as the ventriloquist aftereffect (VAE). Whereas VE most likely indicates an immediate interplay of visual and auditory stimulus processing, VAE most likely reflects a recalibration of spatial auditory representations (i.e. sensory learning). Based on previous ERP-studies, it has been suggested that this recalibration occurs in low-level auditory areas. In the present study, we aimed to identify the neural underpinnings of VAE in humans using fMRI. During fMRI-data acquisition, blocks of unisensory auditory stimuli were presented, separated by blocks of spatially incongruent audiovisual stimuli (A-pretest, AV-recalibration, A-posttest). Sounds were presented from one of three speakers (left, middle, right). During the unisensory auditory pre-/posttests, participants judged where they had perceived each tone via button press. During the audiovisual recalibration blocks, visual stimuli were constantly displaced by 14° to the right of the auditory stimuli and the participants' task was to react to occasional deviant stimuli. The behavioral results revealed that the VAE was successfully induced, i.e. auditory localization judgements were significantly biased to the right in posttests vs. pretests. With regard to the fMRI results, we hypothesized that spatial hearing would be reflected by a difference in neural activity between left- and right-hemispheric auditory areas for lateralized sounds. This difference was expected to be altered by the audiovisual recalibration. Corroborating our hypothesis, we found reduced fMRI-signals in the right auditory cortex (planum temporale, PL) following audiovisual recalibration, replicating previous findings on VE. Importantly, during VAE we additionally observed enhanced fMRI-signals in left auditory cortex (PL), not observed during VE. Moreover, the areas modulated by VAE overlapped with those areas that differentially responded to lateralized sounds in the pretest. Thus, spatially misaligned audiovisual input modulates the differential response in left- and right-hemispheric auditory areas that code for auditory space, and the increase in contralateral neural activity in auditory cortex might be directly related to VAE but not VE.

Disclosures: B. Zierul: None. **B. Röder:** None. **C. Tempelmann:** None. **P. Bruns:** None. **T. Noesselt:** None.

Poster

697. Cross-Modal Processing: Spatial Factors

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Topic: D.03. Multisensory Systems

Title: Interactions between visual capture and front-back confusions in sound localization

Authors: C. MONTAGNE, *Y. ZHOU;
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Abstract: Unlike rodents, primates experience frontal vision while hearing in a 3D spatial domain. Modality-specific attraction and avoidance is crucial for minimizing localization errors during 3-D navigation. As a result of ambiguities in interaural time and level cues, which is known as the "cone of confusion", front-back errors are often reported in sound localization tasks. In this study we investigated the extent to which front-back confusion may be modified by visual stimulation in the frontal field. We are particularly interested in whether or not vision influences the perceived location of a sound source that is outside the field of vision. We had human subjects lateralize 15-ms noise bursts in free field conditions. Two pairs of hidden loudspeakers were positioned in front of and behind a subject at lateral positions of ±45º. Apparent sound source locations spanning 360º around a subject were generated by varying the time delay (up to 1 ms) or the intensity ratio between any two loudspeakers. Three high-powered LED lights positioned in front at -45º, 0º and +45º were used as visual stimuli. Listeners reported their perceived spatial locations of a sound by pushing a button on a touch screen in a single-interval forced-choice experiment. Results show that in audio only (AO) trials subjects show a similar extent of front-back confusion for sound sources in the front and rear hemi-field. In audio-visual (AV) trials front-back confusion decreased (7%) for frontal auditory stimuli and increased (19%) for rear auditory stimuli. Left-right hemifield-specific visual capture also occurred in the AV trials, in congruence with previous "ventriloquist effect" studies. Overall, visual capture is not limited to interactions between information from single light and sound sources, but also extend to perceptual dimensions within an internally computed auditory space. These results suggest that the spatial alignment between vision and audition may fundamentally change the way in which visual cues interact with the directional information from multiple sound sources based on whether they are "in-sight" or "out-of-sight" targets. While visual cues help minimize front-back errors for "in-sight" sound sources by pulling perceived sound locations to the frontal field, visual capture may jeopardize detection of rear-approaching targets.

Disclosures: C. Montagne: None. **Y. Zhou:** None.

Poster

697. Cross-Modal Processing: Spatial Factors

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Support: CONACYT Scholarship 313254

Title: Landmark processing by retrosplenial and postsubicular head direction cells

Authors: *Y. R. LOZANO, P. I. JACOB, M. A. VALENCIA-GARZA, K. J. JEFFERY; Univ. Col. London, London, United Kingdom

Abstract: Spatially modulated neurons in the rat brain such as place, grid and head direction (HD) cells cooperate to form a representation of the local environment that can be used for navigation. A number of studies have examined the role of visual cues in updating the position and directional firing of such cells. However, a question that remains relatively unexplored is how visual information is transformed between primary visual cortex and the spatial system. To investigate this question, extracellular recordings of HD cells were conducted in adult male Lister Hooded rats implanted in the postsubiculum (PoS) and the retrosplenial cortex (RSC). These two interconnected brain regions receive visual and self-motion information from diverse pathways and have been hypothesised to play an important role in the encoding of landmarks. Cue control experiments were conducted as the rats foraged for food inside a light-grey cylindrical arena with two opposing cue cards attached to the inner wall, 180 degrees apart. The cue card pair varied in contrast (black-white), orientation of a bar (vertical-horizontal), height of a bar (top or bottom of the card) and lateral position of a bar (left or right), with control stimuli comprising identical cues. The cues were designed to explore to what extent the cells can process fine details of visual stimuli; if they could not, the cues would be indistinguishable and the cells should either flip randomly between two opposing firing directions within the arena, or else disregard the cues. The cue control protocol consisted of a series of trials where both cue cards were rotated together by a variable magnitude and direction before the rat was placed back in the arena at randomly chosen entry points, and HD cells recorded as it foraged for food. Circular kernel density estimates of preferred firing direction shifts showed that both RSC and PoS HD cells could discriminate the cue cards, as shown by the cells almost always adopting a consistent firing direction within the arena relative to the cue pair. Contrast and orientation features exerted strong landmark control, while height (top-bottom) and symmetry (left-right) were less well discriminated, albeit only slightly. Landmark control for the different cue types did not differ between brain areas suggesting that the activity of HD cells is tightly coupled in the HD cell circuit, consistent with attractor network models. These findings indicate that HD cells can process low-level visual features, and offer a potential model system with which to explore how the signal is transformed from retina through visual cortex to the landmark-processing HD areas.

Disclosures: Y.R. Lozano: None. **P.I. Jacob:** None. **M.A. Valencia-Garza:** None. **K.J. Jeffery:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 698.01/O19

Topic: D.04. Vision

Support: BBSRC BB/L007770/1

Title: A potential extrastriate locus for adaptation to composite radial frequency patterns

Authors: *S. J. LAWRENCE[1], B. D. KEEFE[1], R. J. W. VERNON[1], A. D. GOUWS[1], H. D. BROWN[1], A. R. WADE[1], D. J. MCKEEFRY[2], A. B. MORLAND[1,3];
[1]Univ. of York, York, United Kingdom; [2]Bradford Sch. of Optometry and Vision Sci., Bradford, United Kingdom; [3]Ctr. for Neurosci., Hull York Med. Sch., York, United Kingdom

Abstract: Adaptation to radial frequency (RF) patterns has been used extensively to interrogate the properties of early level shape encoding mechanisms. However, the neural locus of these mechanisms is unclear. Extrastriate visual areas, such as V4, have been hypothesised as likely candidates (Wilkinson et al., 1998; Pasupathy & Connor, 2001; Poirier & Wilson, 2006) and recent TMS evidence has shown area LO2 plays a causal role in the detection of amplitude modulations of RF patterns (Silson et al., 2013). We measured shape aftereffects from composite RF patterns where the phase of one RF component was the adapted feature. We explored the size and location tuning of these aftereffects to gain insight to the receptive field properties that underpin this shape adaptation. We had participants adapt to shape comprising a composite of RF2 and RF3 patterns. The phase of the RF3 pattern in the adaptor was rotated so the shape was asymmetric and appeared similar to the outline of a face with a viewpoint rotated away from the observer. Following adaptation we had participants make judgements on the direction of rotation of a test stimulus. The test stimulus was the same RF2+3 pattern where a staircase procedure was used to establish the phase of the RF3 component that was perceived as front-facing or symmetric. Strong aftereffects were observed for test stimuli that were of equal size and location to the adaptor and corresponded to ~70% of the phase rotation used in the adaptor. The tuning functions were relatively broad for size with aftereffects dropping to ~50% of the normal aftereffect when the size of the test stimulus was half or twice the size of the adaptor. For location, tuning appears to be narrower. Test stimuli presented in the same hemifield as the adaptor but in a different, non-overlapping location elicited only small aftereffects (~20% of the normal aftereffect). The results indicate that the illusion is underpinned by mechanisms that are relatively tolerant to size changes, but less tolerant to changes in location. Given the quantitative relationships we have measured we propose that an extrastriate rather than striate locus for this

shape adaptation is likely. References Pasupathy, A., & Connor, C. E. (2001). Journal of Neurophysiology, 86(5), 2505-2519. Poirier, F. J. A. M., & Wilson, H. R. (2006). Vision Research, 46(15), 2443-55. Silson, E. H. et al. (2013). Nature Neuroscience, 16(3), 267-9. Wilkinson, F., Wilson, H. R., & Habak, C. (1998). Vision Research, 38(22), 3555-68.

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Poster

698. Adaptation and Plasticity in Visual Cortex

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Support: NHMRC APP1066588

ARC CIBF

Title: Adaptation-induced changes in gain, but not correlation, account for perceptual aftereffects

Authors: *N. S. PRICE, E. ZAVITZ, H.-H. YU, M. G. ROSA;
Physiology, Monash Univ., Clayton, Australia

Abstract: Prolonged exposure to a stimulus induces changes in the neuronal responses to subsequent stimuli and changes in the perception of those stimuli. Responsivity and tuning of single sensory neurons have been shown to adapt to reflect stimulus history, and perceptual shifts result when these changes occur across an ensemble of the neural population. We set out to answer two questions: How do neurons, as units and as members of a population, adapt in response to continually changing stimuli; and how does this adaptation produce perceptual phenomena such as the direction aftereffect? We used a multielectrode Utah array to measure the activity of a population of MT neurons in three sufentanil-anaesthetised marmoset monkeys. We presented a continuous motion stimulus consisting of a random sequence of 500 ms periods of coherent motion in one of 12 directions. Each motion period served as both a test and as an adaptor for the subsequent motion periods. As the direction was continuously changing, we defined 144 trial types, representing the sequential pairings of every possible adapt and test direction. This allows us to determine how the adaptation affects both the representation and decoding of the test. With this paradigm, we were able to show for the first time that changes in

neuronal gain and correlation structure outlast the duration of a test stimulus, and can survive intervening visual stimulation (up to three intervening stimuli, or 2 seconds). We used a decoding model to estimate motion direction from 30 ms of neural activity across 20 units. We trained the decoder using neural responses from the adaptation period of 80% of trials and then applied the decoder to the test period (500 ms later) of the remaining 20% of trials. The model gives a continuous (i.e. not categorical) estimate of motion direction. Critically, errors in the predicted test direction depend systematically on the difference between the adaptor and test directions. This systematic dependence was repulsive, as in the classic psychophysical direction aftereffect. For example, an adaptor 60° counterclockwise (positive) from the test produced an error distribution with a mean of 7° clockwise (negative). By training and testing the model on both shuffled and unshuffled data we were able to demonstrate that the changes in responsiveness, not changes in correlation structure were sufficient to produce the direction aftereffect.

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Poster

698. Adaptation and Plasticity in Visual Cortex

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Title: Stimulus dependence of receptive field organization in the visual wulst of owls

Authors: J. MACHADO DE SOUSA[1], P. VIEIRA[1], C. MONIER[2], M. PANANCEAU[2], Y. FRÉGNAC[2], *J. BARON[1];
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Abstract: Accumulating evidence suggests that the receptive field (RF) ON/OFF organization of striate cortex (V1) neurons is not fixed, but varies depending on how sensory input statistics activate different functional topologies of thalamocortical and intracortical connections. This

study was designed to investigate whether such an adaptive feature could also be evidenced in the owl visual wulst, an interesting avian model system to be compared with mammalian V1 since both structures seem to transform incoming signals from the visual thalamus in an analogous manner. Experiments on awake burrowing owls (Athene cunicularia) and lightly sedated barn owls (Tyto alba) were performed using standard single-unit recording techniques. RF subfield preferences for point-light increments (ON) and decrements (OFF) were first measured by reverse correlation with sparse white noise stimuli consisting of pseudorandom sequences of white (112 cd/m2) and black (1 cd/m2) squares (0.3° X 0.3°) presented singly on a grey background (56 cd/m2) for 50 ms. We found that statistically robust spatial activation maps of ON and OFF subfields start approximately 30 ms after stimulus onset and last for about 50 ms. Peak OFF responses (median = 60 ms, interquartile range = 40 - 80 ms) usually occur earlier than their ON counterparts (median = 70 ms, interquartile range = 50 - 90 ms), but no obvious ON/OFF signal strength imbalance was evident across our cell population. The spatiotemporal RF slice exhibiting the largest ON/OFF activity integral was chosen to quantify the degree of overlap between subfields using several indices as in Mata and Ringach (J. Neurophysiol., 93: 919-928, 2005). Strong significant correlations were obtained among all overlap indices, but none of the later could predict the F1/F0 ratio estimated from steady-state responses to optimal sine-wave gratings as in Skottun et al. (Vision Res., 31: 1079-1086, 1991). Sparse-noise RF maps were also found to be clearly distinct to those generated in response to ternary dense noise. The spatiotemporal reorganization of ON/OFF subfields induced by this stimulus statistics modification is compatible with the inverse relationship between RF linearization and noise sparsity found in cat V1 by Fournier et al. (Nature Neuroscience: 14,1053-1062, 2011). Altogether, our results validate the white-noise reverse correlation approach to characterize RF structure dynamics in the owl visual wulst. They further suggest that, like in V1, wulst cells can adapt their linear/non-linear filtering properties to visual context, presumably due, at least in part, to network-regulated mechanisms optimized for natural vision.

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Poster

698. Adaptation and Plasticity in Visual Cortex

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Support: NIH Grant EY09314

Title: Adaptation-induced tuning shifts in excitatory and inhibitory neurons of primary visual cortex

Authors: *D. J. THENGONE, Y. YU, E. I. NITZANY, J. D. VICTOR;
Brain and Mind Res. Inst., Weill Cornell Grad. Sch. of Med. Sci., New York, NY

Abstract: The brain employs a variety of strategies to adjust to changes in the external world. Adaptation is one such strategy, as it modifies responses based on recent inputs. As with other modulatory influences (such as attention) that affect network dynamics, adaptation is likely to involve a broad range of processes at the neuronal level. Here we study how adaptation influences neuronal responses in functionally distinct cortical cell-categories (excitatory vs inhibitory) in two hierarchically-related brain regions, V1 and V2, of the visual pathway. We examined responses of 36 neurons in V1 and V2 of 2 macaques, under propofol/opiate anesthesia and neuromuscular blockade. We performed multi-tetrode single-unit recordings to measure neural responses to drifting sinusoidal gratings before and after 400ms adaptation to preferred and non-preferred stimuli, and we distinguished functionally distinct cell types on the basis of the shape of their extracellular action potentials and a comparison to a much larger laboratory database. We identified a subset of narrow-spiking neurons (putative inhibitory interneurons) that showed narrow orientation tuning curves and small receptive fields, consistent with the properties of parvalbumin-positive interneurons. In both excitatory and inhibitory cell types, we found adaptation-induced changes consisting of gain reduction and tuning shifts. Consistent with previous studies, we found that the shifts could be attractive or repulsive; the wave-shape analysis here reveals that both kinds of shifts are present in both excitatory and inhibitory cell types. These findings have implications for models of orientation selectivity, in which recurrent intracortical connections enhance the orientation bias of incoming thalamic signals. If the tuned recurrent signals are purely excitatory (i.e., in a network in which inhibition is untuned), the model accounts for adaptation-induced reductions in response amplitude and repulsive shifts of the orientation tuning curve. Critically, this type of model does not account for attractive tuning shifts. However, if the recurrent signals also originate from tuned inhibitory neurons - and these inhibitory neurons adapt, as we found -- the model not only predicts the gain reductions and repulsive shifts in the tuning curve, but also accounts for attractive tuning shifts towards the adapted stimulus. More broadly, our findings demonstrate that inhibitory neurons play a key role in the way that sensory representations are recalibrated according to context.

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Poster

698. Adaptation and Plasticity in Visual Cortex

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Topic: D.04. Vision

Support: NSERC

FRQ-NT

Université de Sherbrooke

Title: Cross-correlation investigation of neurons in supra and infragranular layers in cat V1 before and following adaptation

Authors: ***N. CHANAURIA**[1], V. BHARMAURIA[1], L. BACHATENE[1], S. CATTAN[1], J. ROUAT[2], S. MOLOTCHNIKOFF[1];
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Abstract: V1 neurons are systematically organised into domains of selectivity known as orientation columns. Each of these columns is divided into supra (upper) and infragranular (lower) layers wherein neurons are highly similar in different properties such as orientation selectivity. V1 neurons are highly plastic as they have an innate property to change their orientation selectivity in response to a non-optimal stimulus called adapter. On adapting, these neurons change their orientation tuning peaks either towards or away from the adapter exhibiting attractive or repulsive shifts (Dragoi et al., 2000; Ghisovan et al., 2009; Bachatene et al., 2012). Recently our lab showed in supra granular layers that following adaptation entire orientation map is re-organised (Bachatene et al., 2015; Cattan et al., 2014) however there are no reports showing how neurons in deeper layers change their orientation selectivity when adapted. Since the cortical column extends down to layer VI we hypothise that neurons not only to a specific layer change, but the whole cortex is reprogramed. To investigate this we simultaneously recorded layer II-III and V-VI neurons in conventionally prepared anaesthetised cats by lowering a multichannel depth electrode in V1. Cross-correlations were computed within and between the spike trains of neuron pairs of layer II-III and layer V to disclose functional connections and observe how adaptation affects this functional connectivity. Our preliminary data shows that adaptation changes the tuning curves of neurons in supra and infragranular layers. The proportion of neuron pairs in supra and infragranular layers exhibiting positive cross-correlations is relatively small. However contrary to that, cells within the same layers show frequent positive cross-correlations. This data may suggest that neurons in supra and infragranular layers shift their orientation tuning in an independent fashion.

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Poster

698. Adaptation and Plasticity in Visual Cortex

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Support: NIH Grant EY016774

Title: Decoding visual stimulus orientation from adapted neural populations

Authors: *T. B. CZUBA, A. KOHN;
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Abstract: Adaptation is a nearly ubiquitous feature of neural circuits. Although much is known about the effects of adaptation on the tuning of visual cortical neurons, a broad gap remains in understanding how adaptation alters population responses and the information they contain. It is also unclear whether the perceptual effects of adaptation can be explained simply by altered encoding, or whether the inappropriate decoding of adapted signals contributes. To address these issues, we measured the responses of populations of neurons in anesthetized macaque primary visual cortex (V1) following adaptation to high-contrast dynamic orientation stimuli. Experiments were designed to address two primary questions: 1) How does adaptation alter the quality of visual information encoded in a neural population? 2) How might a system alter the readout mechanism(s) to accommodate the effects of adaptation? We adapted neurons with a rapidly presented sequence of drifting gratings (~6° diameter, 80 ms/orientation, 2.4 s/trial). We varied the composition of orientations in the adaptation sequence, from a single orientation to a uniform distribution (0-180°). Brief test stimuli were presented after each adapter. These consisted of small drifting gabors (2.5° fwhm, 400 ms) of different orientations, embedded in dynamic pixel noise. We adjusted the pixel noise on the test stimuli to provide both easily discriminable and more challenging cases. Adaptation had a pronounced effect on V1 population responsivity. We quantified the information about stimulus orientation in the population by measuring the performance of a linear classifier (SVM). Across adaptation ensembles, we found a substantial loss of information about stimulus orientation, relative to an unadapted baseline. Decoder performance decreased more after adaptation to a single orientation than to a uniform distribution of orientations. We tested whether the detrimental effects of adaptation could be overcome by re-training the classifier on the adapted responses. Surprisingly, in most cases performance deficits were not mitigated by re-training. Our results suggest that adaptation reduces information about stimulus orientation in V1, and that this loss of information cannot be compensated for by using a decoder aware of the altered V1 responses. Ongoing experiments in

awake primates performing a fine discrimination task will allow us to relate these findings to perceptual decision making, on a trial-by-trial basis.

Disclosures: T.B. Czuba: None. **A. Kohn:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

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Topic: D.04. Vision

Title: Neural adaptation in primary visual cortex of awake primates

Authors: *M. ALIKHANI[1], A. RAHIMABADI[1], H. RAHIMI NASRABADI[1], M. ZANGANE[1], V. DAVOODNIA[1], R. LASHGARI[1,2,3];
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Abstract: Sensory adaptation is an important physiological phenomenon that results from repeated stimulation of sensory neurons in the brain but whose mechanisms remains poorly understood. Here, we measured the effect of visual adaptation on the spontaneous activity of single neurons in the primary visual cortex of awake behaving primates. We used chronically-implanted ultra-thin electrodes with impedances of 1 to 3 MΩ (Swadlow et al, 2005; Lashgari et al, 2012) in area V1 of awake primates to measure local field potentials (LFPs) and the activity of neighboring single-unit (SU) simultaneously recorded with the same electrode tip. We measured neuronal responses before and after stimulation with a grating drifting at the preferred orientation for 2-3 sec at 2 Hz (usually 4 trials). We analyzed 109 single neurons and found 55 neurons that had a significant reduction in spontaneous activity following stimulation with the preferred stimulus orientation (mean reduction in firing rate: 7.5 SPK/sec; $p<0.001$, Sign rank test). To estimate the cortical depth of the recordings, we measured the polarity of the LFP integrated between 0 and 60 ms following the stimulus onset (Lashgari et al, 2012; Li et al, 2014). We found that reductions in spontaneous firing rate after adaptation could be demonstrated in cortical layers with both positive and negative LFP polarities. To investigate the types of neurons that were most affected by sensory adaptation, we measured the width of spike waveform from the spike onset (initial deflection from baseline) to the maximum amplitude. We found that reductions in the spontaneous firing rate with sensory adaptation could be demonstrated in neurons with different spike widths. We conclude that sensory adaptation can

cause a reduction in the spontaneous firing rate of neurons in the awake primary visual cortex and that this reduction affects different types of neurons located in different cortical layers.

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Poster

698. Adaptation and Plasticity in Visual Cortex

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Gatsby Charitable Foundation

Title: Adaptation to orientation statistics in the visual cortex of awake mice

Authors: ***M. PACHITARIU**, C. STRINGER, M. CARANDINI, K. D. HARRIS;
Univ. Col. London, London, United Kingdom

Abstract: Animals immersed in their natural environments receive a continuous stream of visual inputs, whose temporal statistics depend on context. How do responses in the visual cortex adapt to these temporal statistics? We sought to answer this question in the mouse, where one can most readily dissect circuits and mechanisms underlying computations. We used 2-photon Calcium imaging to monitor neuronal populations in the superficial layers of awake mouse visual cortex, while showing gratings flickering at different temporal rates and modulating the probability of individual orientations. To capture the results we ran simulations of recurrent network models with short-term spike rate adaptation. We obtained good quality receptive fields and orientation tuning curves both for fast and slow stimulus presentation rates, approaching orientation selectivity indices of 1. Responses measured at high presentation rates (10 Hz) were roughly proportional to those measured at 1 Hz, though they were markedly smaller in size. To investigate the effects of bias in the temporal statistics, we increased the probability of presenting one orientation over the remaining orientations. A similar design has been shown to result in cell-specific and stimulus-specific adaptation in the visual cortex of anesthetized cats (Benucci et al, Nat Neurosci, 2013). We found similar results in the awake mouse cortex: stimulus responses were suppressed (contrast adaptation) and tuning curves shifted away from the adapted

orientation (pattern adaptation). The impact of adaptation was broader than the tuning curve of single cells, reducing responses also of cells that did not respond to the adaptor (indirect adaptation). A simple recurrent network of excitatory neurons replicated many of the observed effects. In the model, contrast, pattern and indirect adaptation arise from a combination of specific excitatory connectivity (i.e. enhanced connectivity between neurons of similar preferred orientation) and short-term spike rate adaptation. These results suggest that changes in the temporal statistics of visual stimuli have multiple effects on cortical response. The effects are present in the mouse visual cortex, where they can be studied at the level of microcircuits and cell types. At the level of computation we find that stimulus-specific adaptation - which has beneficial effects for discriminability - could arise from a combination of single cell adaptation and specific recurrent connectivity known to exist in cortex.

Disclosures: **M. Pachitariu:** None. **C. Stringer:** None. **M. Carandini:** None. **K.D. Harris:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 698.09/O27

Topic: D.04. Vision

Title: Short-term light or pattern but not orientation deprivation alters interocular balance in adult macaque visual cortex

Authors: *D. Y. TS'O, M. BEGUM;
Dept of Neurosurg., SUNY - Upstate Med. Univ., Syracuse, NY

Abstract: Short-term monocular deprivation (STMD, patching one eye for 1-3 hours) disrupts interocular balance in adult humans, as measured psychophysically, and also in our present studies using anesthetized adult macaques, as measured with intrinsic signal optical imaging of the V1 ocular dominance columns. Surprisingly, in all these studies, the relative contribution of the patched eye was elevated for more than an hour after patch removal. V1 imaging studies using pattern deprivation rather than full occlusion STMD also yielded a similar rapid shift in interocular balance in which the contribution of the nondeprived eye showed a steady decrease during the deprivation of the other eye. Once the deprivation period ended, the nondeprived eye dramatically shifted course (increased), despite the stimulus to this eye remaining constant during the entire experiment. To further probe the underlying mechanisms of the STMD effect, we tested "orientation deprivation". As with the pattern deprivation studies, the stimuli were

dichoptically viewed 4 degree gratings of various orientations. However during the 1-3hr deprivation period, one eye was presented with only a single orientation (instead of a mean gray screen). The imaging data collected showed no shift in interocular balance to STMD using orientation deprivation. We also conducted multi-electrode recordings in V1 with the STMD paradigm. Single cell responses to monocular and binocular stimuli were plotted before, during and after STMD. The single-unit recordings revealed several different types of cell responses, including cells that showed unremarkable shifts in responses due to the STMD, other cells that exhibited an apparent strengthening of the non-deprived eye during STMD, and cells where the non-deprived eye showed dramatic drops in responsivity during STMD, consistent with the observed imaging (and psychophysical) results. The weakened response for the non-deprived eye during the deprivation period was striking. It cannot be explained by adaptation or fatigue in the eye or cortex. The apparent lack of orientation tuning of the STMD effect suggests a gain-controlling source that is not orientation-tuned. The electrophysiological results show a corresponding alteration of interocular balance at the single cell level. These results matches the previous psychophysical and imaging studies, and suggests a dynamic mechanism for regulating interocular balance and gain that includes the neurons in V1.

Disclosures: D.Y. Ts'o: None. **M. Begum:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

Support: NIH Grant R01EY019466

NIH Grant R01MH091801

Title: Neural mechanism of reactivation of consolidated visual perceptual learning revealed by the concentration of excitatory and inhibitory neurotransmitters

Authors: *J. BANG, K. SHIBATA, T. WATANABE, Y. SASAKI;
Brown Univ., Providence, RI

Abstract: Visual perceptual learning (VPL) by training on a task is interfered with by another training if it occurs within one hour after the initial training. This indicates that VPL is fragile immediately after training but is stabilized within one hour so that it becomes resilient against

interference. However, already stabilized VPL of a task becomes susceptible to interference by practicing a small number of the task again (Bang et al, 2013, VSS). Although this so-called reactivation has made us reconsider a role of stabilization/consolidation in VPL, the neural mechanism has yet to be clarified. Here, we examined excitatory and inhibitory processing associated with reactivation. There were psychophysical and brain-imaging experiments. The psychophysical experiment lasted 3 day. On Day 1, subjects were trained on a detection task (2IFC) on orientation A with 16 blocks in staircase. On Day 2, subjects were asked to perform 3 blocks on orientation A for reactivation and then were trained on orientation B. On Day 3, performance on orientations A and B was measured. In the bran imaging experiment, using functional magnetic spectroscopy, we measured the concentration of glutamate and GABA in the early visual cortex before, immediately after and 3.5 hours after the reactivation. An E(excitation)/I(inhibition) ratio in the early visual cortex was taken by dividing the concentration of glutamate by that of GABA. Performance on orientation A significantly decreased between Days 2 and 3, indicating that reactivation makes already stabilized/consolidated VPL of orientation A labile to interference again. The E/I ratio increased immediately after reactivation of VPL of orientation A, and then returned to the baseline level 3.5 hours later. This time course changes are similar to those for stabilization after the first training (Shibata et al, 2015, VSS), suggesting that the neural mechanism of reactivation is similar to that of stabilization.

Disclosures: **J. Bang:** None. **K. Shibata:** None. **T. Watanabe:** None. **Y. Sasaki:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

Location: Hall A

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Topic: D.04. Vision

Support: NIH Grant EY016774

NIH Grant EY023926

Title: Visual adaptation modulates correlated activity within and between cortical areas

Authors: ***C. A. HENRY**, A. KOHN;
Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Visual processing involves neuronal populations distributed within and between distinct cortical areas. Correlated responsivity in these populations can provide insight into

circuit architecture and the signaling between networks, and may also limit encoded information. Sensory adaptation can modulate this correlated activity; however, recent work has provided conflicting views of its effect on 'noise' correlations in local populations. In addition, it remains unknown how adaptation affects inter-areal correlations and how change in correlations with adaptation depends on the test stimuli. We examined the effect of adaptation on correlations within and between cortical areas, using simultaneous multielectrode recordings in primary visual cortex (V1) and area V2 of anesthetized macaque monkeys. Drifting sinusoidal gratings were presented at a range of test orientations; stimuli were centered and sized to simultaneously drive all V1 and V2 receptive fields. Adaptation consisted of brief (0.4-1.6s) presentations of high contrast gratings immediately prior to the onset of test stimuli. Control (unadapted) responses were measured to test stimuli following an equal period of mean grey screen. Adapted and unadapted trials were randomly interleaved. We found that correlations were modulated by visual adaptation in a stimulus-specific manner. After adaptation, correlations increased for responses to test stimuli similar to the adapter, and either decreased or showed no change for stimuli different from the adapter. This pattern held for both within-area (V1-V1, V2-V2) and across-area (V1-V2) correlations. In contrast, the effect of adaptation on correlations exhibited little dependence upon the orientation preferences of the neuronal pair. To elucidate the mechanisms that might underlie these effects, we measured how correlations and their modulation by adaptation depended on the normalization signals received by the pair. The recruitment of normalization signals has been shown to affect the strength of correlations, and to influence the effects of adaptation on neuronal tuning. We found that neuronal pairs with strong within-receptive field (RF) normalization (i.e. showing strong masking by an orthogonal stimulus) had lower noise correlations than those with weak normalization, both within area (V1-V1) and across areas (V1-V2). However, it was not these neurons, but instead those with weak within-RF normalization, that showed the largest increase in correlations after adaptation. Our results suggest that adaptation affects correlations in a stimulus-specific, not neuron-specific, manner, altering coupling with downstream networks.

Disclosures: C.A. Henry: None. **A. Kohn:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 698.12/O30

Topic: D.04. Vision

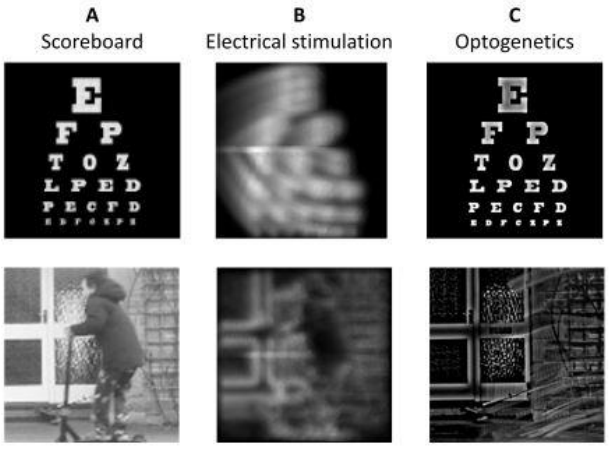
Support: R01EY-014645

R01EY-12925

Title: Pulse trains to percepts: The challenge of creating a perceptually intelligible world with sight recovery technologies

Authors: *I. FINE, G. M. BOYNTON;
Dept. of Psychology, Univ. of Washington, Seattle, WA

Abstract: An extraordinary variety of sight recovery therapies are either about to begin clinical trials, have begun clinical trials, or are currently being implanted in patients. However, as yet we have little insight into the perceptual experience likely to be produced by these implants. Here we focus on methodologies such as optogenetics, small molecule photoswitches, and electrical prostheses, which use artificial stimulation of the retina to elicit percepts. For each of these technologies the interplay between the stimulating technology and the underlying neurophysiology is likely to result in distortions of the perceptual experience. Three example models of the potential perceptual experience of sight recovery are shown. (A) Scoreboard model. The luminance of the percept is linearly related to the strength of current on the retina. (B) Simulation of electrical stimulation. This particular simulation is based on a model of simultaneously stimulating ON- and OFF pathways, followed by the model of axonal stimulation (C) Simulation of small molecule photoswitch stimulation. This simulation is based on the model of simulating ON-centre pathways in isolation, followed by the effects of sluggish temporal dynamics. As well as describing some of these potential distortions, we discuss how they might be minimized either through changes in the encoding model or through cortical plasticity.



Disclosures: I. Fine: None. **G.M. Boynton:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

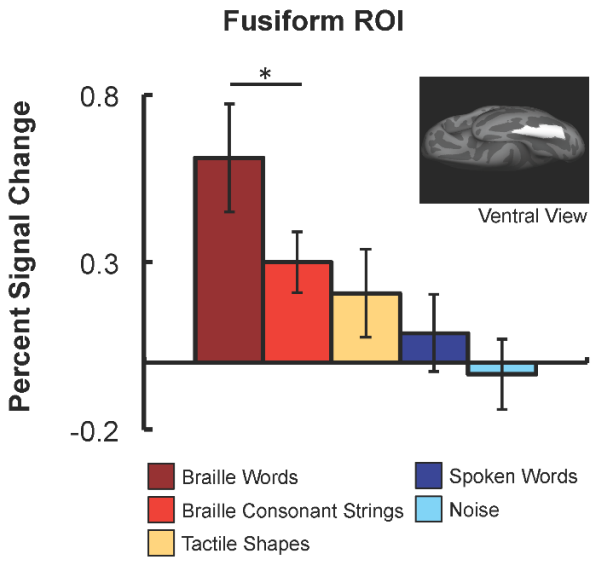
Program#/Poster#: 698.13/O31

Topic: D.04. Vision

Title: Braille processing in visual cortex of congenitally blind individuals

Authors: S. KANJLIA, *J. S. KIM, M. BEDNY;
Johns Hopkins Univ., Baltimore, MD

Abstract: Reading print depends in part on a specialized cortical circuit that supports letter and word recognition, the visual word form area (VWFA). This “reading” area responds more to written language than spoken language or other visual images (Cohen & Dehaene, 2004). Blind individuals read using a tactile system called Braille. Does Braille reading similarly depend on specialized cortical mechanisms? Previous work suggests that like print reading, reading Braille recruits the VWFA (Reich et al., 2011). However, there are alternative explanations for Braille responses in the VWFA relating to language and tactile processing. We used fMRI to ask whether there are cortical mechanisms that process Braille orthography as opposed to spoken language or fine-grained tactile discrimination. We compared reading of Braille words and Braille consonant strings to discrimination of tactile shapes made out of Braille dots, listening to words, and listening to noise (backwards speech). Ten congenitally blind participants heard or felt a list of 6 items followed by a probe item and determined whether the probe came from the preceding list. Regions in ventral, lateral and medial occipital cortices were more active when reading Braille words than listening to auditory words or discriminating tactile shapes. ROI analysis revealed that these occipital areas respond most to Braille words, followed by auditory words and Braille consonant strings, and then tactile shapes and noise. This suggests an interaction between input type (tactile/visual) and linguistic content (language/non-language). In summary, 1) processing of Braille orthography is not restricted to the VWFA but is distributed across regions in visual cortex and 2) unlike the VWFA in sighted adults, Braille areas respond similarly to spoken words and written (Braille) consonant strings, suggesting an influence of high-level linguistic information. We conclude that blind individuals recruit visual cortex for orthographic processing, but visual cortex Braille reading mechanisms are qualitatively different from those for reading print.



Disclosures: **S. Kanjlia:** None. **J.S. Kim:** None. **M. Bedny:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 698.14/O32

Topic: D.04. Vision

Support: Fight for Sight Project Grant

Title: Can an fMRI signature of reorganization of visual processing in patients with retinal lesions be found in normally sighted individuals?

Authors: ***H. D. BROWN**[1], A. D. GOUWS[1], R. GALE[2], S. J. D. LAWRENCE[1], R. J. W. VERNON[1], H. A. BASELER[1,3], A. B. MORLAND[1,3];
[1]Psychology, Univ. of York, York, United Kingdom; [2]York Teaching Hosp. NHS Fndn. Trust, York, United Kingdom; [3]Ctr. for Neurosci., Hull York Med. Sch., York, United Kingdom

Abstract: Macular degeneration (MD) causes a loss of central vision, removing input to the corresponding representation in primary visual cortex (V1) referred to as the Lesion Projection Zone (LPZ). There is disagreement in the literature as to whether the LPZ can be activated, and under what conditions such activity can be detected. Moreover, it has been debated whether activity in the LPZ can be taken as evidence of reorganization. For example, Baker et al. (2005)

reported activity in the LPZ during a stimulus-related task and interpreted it as a reorganization of visual processing. Masuda et al. (2008) found similar activity in the LPZ, but only during stimulus-related task conditions, and not passive viewing. This was taken to support the view that feedback from extrastriate areas is responsible for activity in the LPZ of V1. Importantly, LPZ activity was only detected in MD patients, but not controls. However, it is challenging to provide an appropriate control condition in normally sighted individuals. In this study we provided a control condition that better mimics the deficit that affects MD patients. To this end we presented a brightly lit (15,000 cdm-2) central 12deg (diameter) disc to participants with normal vision, thereby bleaching central retina to generate a transient 'retinal lesion'. An fMRI block design experiment was performed comparing responses to faces or scrambled faces with responses to a mean gray background. Participants were exposed to the central bleaching stimulus at the beginning of the scan and topped up for 18s between 6s stimulus or gray intervals. Stimuli subtended 5x5deg and were presented in the upper right quadrant adjacent to the bleached area. Scans were repeated in both passive viewing and one-back task conditions. The simulated LPZ and surrounding stimulus representation were localised in each participant in separate scans. Analysis of the BOLD responses to the visual stimuli during passive viewing revealed that activity was elicited at the stimulus representation and did not spread into the representation of the bleached zone. In contrast, activity spread into the representation of the bleached zone when participants performed a stimulus-related task. Our results indicate that one of the response signatures that has been taken as evidence for reorganization can be observed in normally sighted control participants. It is unlikely therefore that mechanisms giving rise to signals in the LPZ are specific to patients, but are more likely a reflection of normal feedback from extrastriate visual areas as proposed by Masuda et al. (2008). Baker et al., J.Neurosci (2005). 25(3):614 - 618. Masuda et al., Cerebral Cortex (2008). 18:2483-2493.

Disclosures: **H.D. Brown:** None. **A.D. Gouws:** None. **R. Gale:** None. **S.J.D. Lawrence:** None. **R.J.W. Vernon:** None. **H.A. Baseler:** None. **A.B. Morland:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 698.15/O33

Topic: D.04. Vision

Support: ERC grant 310809

McDonnell grant 220020284

Title: Exploring the preservation of specificity in 'visual' areas of the human congenitally blind brain using the EyeMusic Sensory Substitution Device and fMRI imaging

Authors: S. MAIDENBAUM[1], S. ABBOUD[1], S. DEHAENE[2], *A. AMEDI[1];
[1]Dept. of Develop. Med. Neurobio., Fac. of Medicine, The Hebrew Univ. of Jer, Jerusalem, Israel; [2]Colle`ge de France, Universite´ Paris 11, Inst. Natl. de la Sante´ et de la Recherche Me´dicale, Paris, France

Abstract: Over the past decade a series of 'visual' brain regions with preferred specificity for certain categories of stimuli have been located and researched. For example, the Visual Word Form Area (VWFA) shows preferential selectivity for the shapes of letters and the Visual Number Form Area (VNFA) shows selective preference for the shape of numbers. But what happens to these regions in congenital blindness? How large a part does vision take in driving and creating this preference? what is the contribution of shape biases to its formation and whether visual processing underlies it? These questions are especially intriguing in the case of categories such as those above, which only became relevant very recently on an evolutionary time scale yet still show anatomical consistency across human brains. How do we have regions dedicated specifically to letters or numbers if we only started using these concepts several thousand years ago? Here we use congenital blindness as a model for brain development without visual experience. During fMRI, we present blind subjects with shapes encoded via The EyeMusic, a novel visual-to-auditory Sensory Substitution Device which can convey whole scene visual information such as location, shape and color via audition. We find that greater activation is observed in the rITG when subjects process symbols as numbers compared with control tasks on the same symbols. Using resting-state fMRI in the blind and sighted, we further show that the areas with preference for letters and numerals exhibit distinct patterns of functional connectivity with language-processing and quantity areas, respectively. These findings suggest that specificity in the ventral ‘visual’ stream can emerge independently of sensory modality and visual experience, under the influence of distinct connectivity patterns. Finally, we will then turn to discuss more low-level visual features such as color - will such features also emerge independently of sensory modality and visual experience? And to what extent will they do so?

Disclosures: **S. Maidenbaum:** None. **S. Abboud:** None. **S. Dehaene:** None. **A. Amedi:** None.

Poster

698. Adaptation and Plasticity in Visual Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 698.16/O34

Topic: D.03. Multisensory Systems

Support: EY-014645 to Ione Fine

K99EY023268 to Fang Jiang

Title: Examining tactile motion responses within hMT+

Authors: *F. JIANG[1], M. S. BEAUCHAMP[2], I. FINE[3];
[1]Psychology, Univ. of Nevada, Reno, Reno, NV; [2]Univ. of Texas Med. Sch. at Houston, Houston, TX; [3]Univ. of Washington, Seattle, WA

Abstract: Here we examine tactile motion BOLD responses within the human MT+ complex. Although several studies have reported tactile responses overlapping with hMT+, many used group average analyses, leaving it unclear whether these responses were restricted to sub-regions of hMT+. Moreover, previous studies either employed a tactile task or passive stimulation, leaving it unclear whether or not tactile responses in hMT+ are simply the consequence of visual imagery. Here we carried out a replication of one of the classic papers finding tactile responses in hMT+ (Hagen et al. 2002). We mapped MT and MST in individual subjects using visual field localizers. We then examined responses to tactile motion on the arm, either presented passively or in the presence of a visual task performed at fixation designed to minimize visualization of the concurrent tactile stimulation. Without a visual task, we found only weak tactile motion responses in MT (6% of voxels showing tactile responses) and MST (2% of voxels). With an orthogonal visual task, responses in MST reduced to almost nothing (<1% regions). Consistent with previous results, we did observe tactile responses in STS regions superior and anterior to hMT+. The weak nature of tactile responses in hMT+ (and their abolition by withdrawal of attention) suggest that hMT+ may not serve as a general, cross-modality motion processing module.

Disclosures: F. Jiang: None. **M.S. Beauchamp:** None. **I. Fine:** None.

Poster

699. Striate Cortex: Response Properties

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

Support: NIH grant R01 EY019743

NSF Grant IOS-1355075

RPB

Title: Parallel processing of center and surround signals in the superficial and deep layers of macaque V1

Authors: *M. BIJANZADEH[1,2], L. NURMINEN[3], A. ANGELUCCI[3];
[1]Ophthalmology, Moran Eye Inst., Univ. of Utah, Salt Lake City, UT; [2]Neurosci. Program, Salt lake city, UT; [3]Moran Eye Institute, Univ. of Utah, Salt lake city, UT

Abstract: The laminar architecture is a fundamental principle of cortical organization. Yet, we still do not know how simple sensory stimuli are processed across cortical layers. Using linear electrode arrays in macaque V1, we have examined the spatiotemporal patterns of laminar activation induced by visual stimuli presented inside and outside the receptive field (RF) of the recorded cells in the column. Small (0.5-1.5°) grating patches were flashed inside the RF, or in the surround at progressively larger distances from the RF. Annular gratings (2° in width) were also flashed in the surround. We recorded both local field potentials (LFPs) and spikes, and applied current source density (CSD) analysis to LFPs. At each cortical depth we measured the onset latency of the first current sink in CSD and of the spiking responses in the PSTHs. CSD in the absence of RF stimulation reflects the net synaptic input caused by connections terminating in a layer. A 0.5° stimulus inside the RF evoked the earliest CSD signals in layer (L) 4C, followed by supragranular (SG) and infragranular (IG) layers almost at the same time. Larger (1-1.5°) gratings inside the RF caused faster onset of CSD signals in L6, almost as fast as in L4C. The earliest spiking activity in response to either stimuli occurred in L4C and IG layers almost at the same time. Small (0.5-1.5°) patch stimuli located up to 2.25° from the RF center (in the near surround) evoked markedly delayed current sinks in L4C. When these stimuli in the surround were collinearly aligned in visual space to the preferred orientation of the recorded cells in the center, current sinks occurred first and almost simultaneously in L6 and SG layers. Instead, non-collinear surround stimuli evoked the earliest CSD signals in L6, but no activation of SG layers. These data suggest that intra-V1 horizontal connections (HC) within SG layers and L6 provide the modulatory signals from the near surround; moreover while these horizontal inputs in the SG layers are specific for orientation and retinotopic axis (consistent with the known functional specificity of HC), horizontal inputs within L6 are not. Surround gratings located ≥3° from the RF center (in the far surround) evoked the earliest CSD signals almost simultaneously in L1 and 6, but markedly delayed current sinks in SG layers. These data are consistent with our previous proposal that inter-areal feedback connections to V1 IG layers and L1 provide the modulatory signals from the far surround. Similar onset latencies of granular and IG activation induced by stimuli in the RF, and of superficial and deep layers activation induced by stimuli in the surround suggest two parallel processing circuits within the cortical column.

Disclosures: **M. Bijanzadeh:** None. **L. Nurminen:** None. **A. Angelucci:** None.

Poster

699. Striate Cortex: Response Properties

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 699.02/O36

Topic: D.04. Vision

Support: NIH Grant RO1 EY019743

NSF Grant IOS-1355075

Research to Prevent Blindness

Title: Anatomical and functional specificity of V2-to-V1 feedback circuits in the primate visual cortex

Authors: *F. FEDERER, S. MERLIN, A. ANGELUCCI;
Moran Eye Inst., Univ. of Utah, Salt Lake City, UT

Abstract: Corticocortical feedback (FB) connections are a prominent feature of the cerebral cortex whose role remains speculative. In the visual cortex FB may mediate functions such as attention and contextual effects. To understand how FB connections perform such computations, it is crucial to understand how they are anatomically and functionally organized. Previous studies of FB connections have been hampered by the unavailability of efficient anterograde tracers that unambiguously label FB axons, without also labeling the reciprocal V1-to-V2 axons. As a consequence, there have been conflicting reports of both anatomically diffuse and non-orientation specific (Stettler et al. 2002), and patchy and orientation-specific (Shmuel et al. 2005) V2-to-V1 FB connections. Here we have examined the anatomical and functional organization of V2-to-V1 FB connections in macaque and marmoset monkeys, using a GFP-expressing AAV9 viral tracer that allows unambiguous labeling of FB axons. Injections were targeted to specific V2 cytochrome oxidase (CO) stripes and orientation-preference domains identified using intrinsic optical imaging (OI). After 5-7 weeks animals were either perfused and their brain sectioned sagittally to identify cortical layers, or underwent further OI of orientation, retinotopy and ocular dominance maps in V1 and V2. The latter were sectioned parallel to the imaged plane, and labeled FB axons warped to the functional maps using surface vasculature. We found that in both primate species, V2 FB to V1 shows laminar and compartmental specialization. Both thick and thin V2 stripes send FB primarily to V1 layers (L) 1-2 and 5, and sparser projections to L3A and 6; however, only thick stripes send projections to L4B. In all V1 layers of termination, V2 FB axons form columnar patches. The patches arising from cells in the V2 thick stripes lie primarily in the V1 interblobs, and those from thin stripes lie primarily in the CO blobs. In

summary, there exist parallel FB pathways from V2 to V1 that target the same V1 layers and compartments giving rise to parallel feedforward projections to V2. We also found that V2 FB axons arising from thick stripes preferentially contact V1 domains with similar orientation preference, showing no bias for left or right eye columns. Moreover, these FB fields are markedly anisotropic in V1, with their retinotopic axis of elongation being collinear with the orientation preference of the V2 injected site. In contrast, V2 FB axons arising from thin stripes predominantly terminate at/near orientation pinwheel centers in V1. Orientation-specific FB pathways may underlie orientation-tuned far-surround suppression (Angelucci et al. 2002).

Disclosures: **F. Federer:** None. **S. Merlin:** None. **A. Angelucci:** None.

Poster

699. Striate Cortex: Response Properties

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 699.03/O37

Topic: D.04. Vision

Support: NIH R01 EY018861

Title: Frontal cortex for top-down control: organization of long-range inputs and outputs

Authors: ***S.-Y. ZHANG**[1], M. XU[2], W. CHANG[3], J. DO[3], Y. DAN[2];
[1]HHMI /UC Berkeley, Berkeley, CA; [2]HHMI/UC Berkeley, Berkeley, CA; [3]UC Berkeley, Berkeley, CA

Abstract: Long-range projections from the frontal cortex are known to modulate sensory processing in multiple modalities. Although the mouse has become an increasingly important animal model for studying the circuit basis of behavior, the functional organization of its frontal cortical long-range connectivity remains poorly characterized. Here we used virus-assisted circuit mapping to identify the brain networks for top-down modulation of visual, somatosensory, and auditory processing. The visual cortex is reciprocally connected to the anterior cingulate area, whereas the somatosensory and auditory cortices are connected to the primary and secondary motor cortices. Anterograde and retrograde tracing also identified other cortical and subcortical structures belonging to each network. Furthermore, using novel viral techniques to target subpopulations of frontal neurons projecting to the visual cortex versus the superior colliculus, we identified two distinct subnetworks within the visual network. These findings provide an anatomical foundation for understanding the brain mechanisms underlying top-down control of behavior.

Disclosures: **S. Zhang:** None. **M. Xu:** None. **W. chang:** None. **J. Do:** None. **Y. Dan:** None.

Poster

699. Striate Cortex: Response Properties

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 699.04/O38

Topic: D.04. Vision

Title: Readout of fine-resolution shape contours from V1 activity via bottom-up reconstruction

Authors: *G. ZURAWEL, I. SHAMIR, H. SLOVIN;
The Leslie and Susan Gonda (goldschmied) Multidisciplinary Brain Res. Ctr., Ramat-Gan, Israel

Abstract: Primates have a remarkable capacity to process visual stimuli under variable conditions. The primary visual cortex (V1) employs multiple mechanisms for encoding the physical stimulus attributes: receptive fields, contrast, non-linearity, etc. In contrast, complex features like invariant categorization and face processing are associated with RF convergence to and processing by higher visual areas. However, the visual experience and perception of objects are rich and detailed, not completely "stripped down" to invariant "labels" only (e.g. invariant category). V1 is a natural candidate facilitator of the "detailed" component of visual representation, yet its actual role remains unclear. To facilitate detailed visual perception, representation of both physical (stimulated) and implied (non-stimulated) stimulus features (e.g. missing contour parts) should be present, and therefore potentially re-constructible from evoked V1 neuronal activity. Reconstructing visual contents directly from cortical data via naïve, bottom-up modeling has had limited success so far. Specifically, extracting object contours in high-resolution has not yet been accomplished. We therefore set out to reconstruct contours of both physical (stimulated) and implied (non-stimulated) parts of stimuli directly from cortical activity and without use of a prior image bank. We presented small monochromatic shapes that varied in shape, size and position, that were either complete or had a missing part of the contour. Using voltage sensitive dye imaging (VSDI) from fixating monkeys, we imaged V1 responses, capturing sub- and supra-threshold membrane potentials of neuronal populations. Reconstruction was carried out by reversal of a simplified, brain-inspired bottom-up encoding model that accounts for retinotopy, receptive fields, contrast and non-linearity. Both physical contours and the "completed" representation of implied contours were reconstructed from the data, including at single-trial level. Overall, we demonstrate physical and implied contour reconstruction directly from V1 cortical activity without the use of an image prior. Our results indicate that V1 serves an important role in facilitating detailed visual representation.

Disclosures: **G. Zurawel:** None. **I. Shamir:** None. **H. Slovin:** None.

Poster

699. Striate Cortex: Response Properties

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 699.05/O39

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Support: NSF Grant 1355075

NIH R01 EY019743

Ella and Georg Ehrnrooth Foundation Postdocoral Fellowship

Title: Natural images and redundancy reduction in primate visual cortex

Authors: ***L. O. NURMINEN**, M. BIJANZADEH, A. ANGELUCCI;
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Abstract: Barlow (1961) suggested that early sensory processing should reduce redundancy between neural responses. Theoretical work by Schwartz and Simoncelli (2001) applied this principle to natural images, and found that cortical non-linearities such as surround suppression are well suited for redundancy reduction. Experimentally, natural stimulation of the receptive field (RF) surround reduces pair-wise correlations between the responses of neurons in primary visual cortex (V1; Vinje & Gallant 2000). However, for highly non-Gaussian signals, such as neural responses to natural images, correlations may be only weakly related to redundancy. Thus, it is unknown whether there are mechanisms in primate visual cortex for reducing redundancy between neural responses to natural images. To address this question, we recorded V1 cell responses using Utah electrode arrays in sufentanil anesthetized macaques. The linear RFs of 41 simple-like single- and multiunits were estimated using binary noise and an automatic locality determination algorithm (Park & Pillow 2011). Spike count responses were measured to repeated (20 times) and randomized sets of monocularly displayed 6º x 6º natural image patches randomly sampled from the Van Hateren image database. The dependencies between the measured spike-count responses were compared to predictions made by the linear RF estimates. RF non-linearities and neural noise estimated from the measured neural responses were added to the linear estimates. Moreover, to elucidate how non-linear RF surrounds affect statistical dependencies, the dependencies in spike-count responses to the 6º x 6º image patches were compared to the dependencies in spike-count responses to the same image patches windowed so

that the minimum response field of just few neurons was stimulated. We found that the correlations between the measured spike count responses were lower than the correlations predicted by the linear RF. Correlations were reduced the most between cells with similar RFs. This reduction originated from both the instantaneous spike-rate non-linearity and surround suppression. In addition, we characterized the higher-order dependencies between neural responses by first removing the correlations between the responses, and then computing energy correlation and mutual information between the responses. We found that also the higher-order dependencies between the measured spike-counts were lower than those predicted by the linear RFs. These results demonstrate that non-linearities in primate V1 reduce redundancy between neural responses to natural images.

Disclosures: L.O. Nurminen: None. **M. Bijanzadeh:** None. **A. Angelucci:** None.

Poster

699. Striate Cortex: Response Properties

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Support: NIH T32 EY018080

NSF BCS1261433

Title: Effects of naturalistic vision on contrast sensitivity and V1 activity

Authors: *J. NIEMEYER, M. PARADISO;
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Abstract: Most studies of visual processing and perception are designed to minimize stimulus complexity and eye movements -- both key components of natural vision. Clinical tests are also generally conducted with simple stimuli lacking context. Yet, research in our lab has shown that saccadic eye movements and scene complexity significantly influence neuronal responses in area V1. Here we examine whether more naturalistic vision impacts contrast sensitivity, an important tool for characterizing vision and visual disorders. While recordings were made from V1 neurons, macaques performed a 2AFC contrast discrimination task. Gabor stimuli were presented, either inside or outside a V1 receptive field (RF), on a complex scene background; the stimuli were either flashed on or brought into an RF by a saccadic eye movement. Contrast sensitivity in the more natural saccade condition was reduced by over 30% compared to flashed

stimulation and V1 population responses were reduced by over 25%. Moreover, there was a clear correlation in the neural and perceptual measures: performance was similar in flash and saccade conditions at higher spatial frequencies (> 6 cy/deg) but at lower spatial frequencies performance was significantly worse with saccades. These findings demonstrate that natural vision has important effects on contrast sensitivity and the effects correlate with V1 visual responses. We hypothesized that the cause of the frequency-dependent differences was rapid adaptation to the portion of the natural image that covers the RF prior to the Gabor test stimulus. In the saccade condition, this adaptation might carry across the eye movement and influence the response to the Gabor at the end of the saccade. In the fixation condition adaptation may wane before the Gabor is flashed on. We found that when we covered the RF with uniform gray prior to the saccade, contrast sensitivity and V1 responses were the same in fixation and saccade conditions. This finding suggests a novel form of rapid adaptation that carries across saccades. Thus, contrast sensitivity and V1 responses are reduced with natural images, which are dominated by low SFs. These findings have broader implications for the accurate characterization of V1 responses and clinical assessment in more naturalistic vision.

Disclosures: J. Niemeyer: None. **M. Paradiso:** None.

Poster

699. Striate Cortex: Response Properties

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IDEX Paris-Saclay

E.C. Human Brain Project (FP7-604102) to AD, YZ

E.C. BrainScales Project (FP7-269921)

Title: Conductance-based interactions predict the suppressive effect of interacting propagating waves in awake monkey visual cortex

Authors: *A. DESTEXHE[1], Y. ZERLAUT[1], S. CHEMLA[2], A. REYNAUD[2], F. CHAVANE[2]; [1]CNRS, Gif-sur-Yvette, France; [2]INT, CNRS, Marseille, France

Abstract: Propagating waves can be detected in primary visual cortex of awake monkey, either in spontaneous activity of following visual stimulation, as recently shown by a phase-based analysis (Muller et al., Nat. Comm., 2014). Using voltage-sensitive dye imaging, we show here that two visual stimuli in different positions in space and time evoke two distinct propagating waves, which can collide. The interaction between the two waves is always suppressive (sub-linear). To investigate possible mechanisms for such a suppressive effect, we used a population (mean-field) model of cortical activity, taking into account excitatory and inhibitory conductance-based interactions. This model used "biologically-realistic" transfer functions based on a recent characterization from slice experiments with dynamic-clamp injection of conductance-based synaptic inputs in cortical neurons. In response to an afferent localized excitatory input, this model reproduced propagating waves arising from lateral connectivity. Interestingly, this model also reproduced the experimental observations of the suppressive interactions between propagating waves. In this case, the suppression was caused by the conductance (shunting) effect of recurrent synaptic interactions. The conductance-based model reproduced the speed of the suppression as seen in the experiments. Using transfer functions without this conductance effect, did not reproduce these features and were inconsistent with experiments. We conclude that conductance-based interactions between recurrent synaptic inputs is a possible candidate explanation for the suppressive effect of propagating waves in visual cortex.

Disclosures: **A. Destexhe:** None. **Y. Zerlaut:** None. **S. Chemla:** None. **A. Reynaud:** None. **F. Chavane:** None.

Poster

699. Striate Cortex: Response Properties

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Support: Gatsby grant GAT3138

Gatsby grant GAT3213

Title: Visually-driven behavior at the limits of sensory information

Authors: ***B. SRIRAM**[1], A. CRUZ-MARTÍN[2], L. LI[1], D. BISHOP[1], A. GHOSH[1,3]; [1]Div. of Biol., UCSD Div. of Biol., La Jolla, CA; [2]Boston Univ., Boston, MA; [3]CNS Discovery and Biomarkers, F. Hoffmann-La Roche, Basel, Switzerland

Abstract: One of the most salient features of neural responses in primary visual cortex (V1) is its preference for stimuli oriented at a specific angle. While V1 orientation preferences are largely shaped by the pattern of thalamic inputs, the way in which these neural representations develop over time and how the individual circuits components contribute to this development is unclear. To understand this dynamics, we have developed a visual discrimination behavior task in freely moving rodents. By forcing mice to make visual discrimination decisions after presenting brief stimuli, we find that mice integrate information extremely quickly and are able to perform above chance with very brief stimulus exposures. Reversible V1 Lesion by activating Channel rhodopsin targeted into PV neurons, causes significant reduction in performance indicating that the task is cortex dependent. Electrophysiological recordings indicate that at these very short stimulus durations there is high failure rate (fraction of trials with no spiking) in cortical firing, indicating that the cortex can compute complex discrimination using extremely sparse data. (This work is supported by the Gatsby Charitable Trust)

Disclosures: **B. Sriram:** None. **A. Cruz-Martín:** None. **L. Li:** None. **D. Bishop:** None. **A. Ghosh:** None.

Poster

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Support: Feodor Lynen Scholarhip from the Alexander von Humboldt Foundation

Title: Decoding orientation of contrast edges from evoked and induced oscillatory brain activity

Authors: M. FANG[1], J. LI[1], Q. LI[3], R. CICHY[1], *D. PANTAZIS[2];
[2]McGovern Inst. for Brain Research, MIT, [1]MIT, Cambridge, MA; [3]Massachusetts Gen. Hosp., Boston, MA

Abstract: To yield a coherent perception of the visual world the human brain must bind simple features into complex wholes. A seminal study by Gray et al. (1989) has shown that spatially separate orientation columns in cat V1 lock their gamma oscillations (40-60Hz) to reflect global stimulus properties, suggesting gamma oscillations as a mechanism for simple feature binding. Since then a multitude of studies has revealed the role of gamma frequencies as mediators of bottom-up feature binding. However, the relationship between evoked and induced gamma oscillatory responses in the encoding of simple visual features is still under debate. To

investigate the encoding of simple visual features in both evoked and induced oscillatory responses we systematically investigated the encoding of oriented contrast edges in coherent wholes (gratings). We recorded human MEG data while participants viewed six grating stimuli with different angles (0-150° in 30° steps; 800 ms presentation time; 3 cycles/degree) (Fig 1a), performed time-frequency Morlet wavelet decomposition, and used multivariate pattern classification on MEG sensor levels to decode stimulus orientation. Our findings show that orientation information can be decoded not only from evoked signals (Fig 1b; significant onset 39ms) but also from oscillatory components with two dominant frequency ranges: 25-30Hz and 50-60 Hz (Fig 1c). Comparing representations of orientation across time (train classifier at one time point, test at other time points), we found that evoked components had transient representations (Fig 1d), whereas oscillatory components were sustained for the duration of the stimulus (Fig 1e,f). Together, our results show that both evoked and induced components encode simple visual features, though their different temporal dynamics indicate they may subserve different neuronal processes.

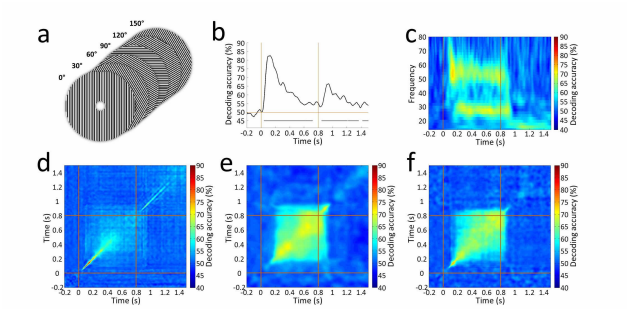

Figure 1. a) The stimulus set consisted of 6 grating patterns with different orientations and randomized phase. We used multivariate pattern classification to decode orientation between pairs of grating patterns. **b)** Orientation information could be decoded from evoked responses both at the onset (0s) and offset (0.8s) of the stimulus. Horizontal line indicates statistical significance. **c)** Orientation information could be decoded from induced responses at two frequency bands (25-30Hz and 50-60Hz). **d-f)** Time-time decoding shows that evoked responses were transient (d) whereas induced components at 25-30Hz (e) and 50-60Hz (f) were sustained.

Disclosures: **M. Fang:** None. **J. Li:** None. **Q. Li:** None. **R. Cichy:** None. **D. Pantazis:** None.

Poster

700. Organization of ExtraStriate Cortex

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JSPS Postdoctoral Fellowship for Research Abroad

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The Indiana University Dean's Fund

Title: Occipital vertical fiber system in human and macaque

Authors: *H. TAKEMURA[1,2], F. PESTILLI[3], K. S. WEINER[4], G. A. KELIRIS[5,6], S. LANDI[7], J. SLIWA[7], F. Q. YE[8], M. BARNETT[4], D. A. LEOPOLD[8], W. A. FREIWALD[7], N. K. LOGOTHETIS[5], B. A. WANDELL[4];
[1]Ctr. for Information and Neural Networks (CiNet), Natl. Inst. of Information and Communicatio, Suita-shi, Japan; [2]Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [4]Dept. of Psychology, Stanford Univ., Stanford, CA; [5]Max Planck Inst. for Biol. Cybernetics, Tübingen, Germany; [6]Bio-Imaging Lab, Dept. of Biomed. Sci., Univ. of Antwerp, Antwerpen, Belgium; [7]The Rockefeller Univ., New York, NY; [8]Natl. Inst. of Hlth., Bethesda, MD

Abstract: The large size of the human brain imposes computational constraints that are reflected in its structural organization. For example, specialized visual processing of spatial and categorical information is partially segregated in dorsal and ventral regions in the occipital and temporal lobes; these regions are widely separated in cortex. For effective vision and action, the processing performed in these regions must be coordinated. Classical as well as recent studies identify the human Vertical Occipital Fasciculus (VOF), as a likely white matter bundle that includes axons that communicate between the dorsal and ventral regions. In this study, we compare the human vertical occipital pathways with similar tracts in the much smaller macaque brain in order to better understand similarities and differences across species. Methods. We obtained diffusion MRI (dMRI) at several spatial resolutions, and we used fiber tractography to estimate the trajectories of several different occipital pathways. DMRI data were acquired from 4 macaque monkeys and many humans (Takemura et al., 2015). We analyzed the data using constrained spherical deconvolution (CSD) and an ensemble of probabilistic tractography methods. We optimized the tractography results and tested statistical hypotheses using Linear Fascicle Evaluation methods (Pestilli et al., 2014). Results. A substantial bundle of vertical occipital fibers could be found in all the macaque and human datasets. The location of the macaque VOF (mVOF) is consistent with a schematic description in a post-morterm monkey brain described by Wernicke (1881). The mVOF terminates near cortical areas V3d, V3A, V4d and MT dorsally and V4v/TEO ventrally. The pattern of mVOF terminations is similar to those of human VOF, which are principally V3A/B dorsally and hV4 ventrally. In both species, the VOF is lateral to the optic radiation (sagittal stratum). In human, the VOF is also lateral to the Inferior Longitudinal Fasciculus (ILF) and clearly distinguishable from the ILF; but the mVOF intermingles with the vertical component of macaque ILF and does not form a very distinct bundle. The estimates of the position, size and cortical terminations of the mVOF depend on

instrumental parameters, such as dMRI resolution; but in all cases the core of the tract can be identified and the estimates are consistent. These findings suggest that the human and macaque vertical occipital fiber systems diverged from a common ancestor. The human system significantly enlarged and became separated from the ILF. The change in white matter may be part of the general evolution of the size and position of extrastriate maps with the increase in brain size.

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Poster

700. Organization of ExtraStriate Cortex

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DFG

Plasticise Consortium (Project HEALTH-F2-2009-223524)

Title: Population receptive field changes in hV5/MT+ of healthy subjects with simulated visual field scotomas

Authors: *A. PAPANIKOLAOU[1,2], G. A. KELIRIS[1,3], S. LEE[2], N. K. LOGOTHETIS[1], S. M. SMIRNAKIS[2];
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Abstract: An important question is whether the adult visual cortex is able to reorganize in subjects with visual field defects (scotomas) as a result of retinal or cortical lesions. Functional magnetic resonance imaging (fMRI) methods provide a useful tool to study the population receptive field (pRF) properties and assess the capacity of the human visual cortex to reorganize following injury. However, these methods are prone to biases near the boundaries of the scotoma. Retinotopic changes resembling reorganization have been observed in the early visual

cortex of normal subjects when the visual stimulus is masked to simulate retinal or cortical scotomas. It is not known how the receptive fields of higher visual areas, like hV5/MT+, are affected by partial stimulus deprivation. Here, we measured responses in human area V5/MT+ in five healthy subjects under two stimulation conditions. FMRI measurements were obtained under the presentation of a moving bar stimulus spanning the entire visual field while the subjects were fixating. In a second session the stimulus was masked in the left upper quadrant of the visual field to simulate a quadrantanopic scotoma ("artificial scotoma" or AS) occurring often as a result of partial V1 or optic radiation lesions. PRF estimates were obtained using a recent method of pRF topography estimation (Lee et al., A new method for estimating population receptive field topography in visual cortex, NeuroImage, 2013) which is consistent with other pRF methods. Responses obtained under the AS condition were compared with simulations obtained from a linear AS model (or LAS model). The LAS model provides an estimation of the pRF changes expected to occur as a result of the truncated stimulus assuming that the pRF linearly integrates the AS. We found that pRFs in hV5/MT+ are nonlinearly affected by the truncated stimulus presented: pRF centers shifted towards the border of the AS, pRF size decreased and pRF amplitude increased near the AS border. In addition, using the full bar stimulus to estimate the pRF topography (when in fact the stimulus presented included the AS) produced erroneous pRF estimates inside the region of the artificial scotoma. These biases are not the result of a trivial methodological artifact but appear to originate partly from asymmetric BOLD responses occurring when the stimulus moves from seeing to non-seeing locations of the visual field. Distinguishing between pRF changes that occur as the result of true reorganization versus different test-stimulus presentation conditions is an important task that needs to be undertaken when studying visual cortex organization in patients with visual field deficits.

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Poster

700. Organization of ExtraStriate Cortex

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Support: Netherlands Organization for Scientific Research (NWO) Vidi Grant 452-08-008 to SD

Title: Deriving contrast response functions from fMRI responses to natural images

Authors: *W. ZUIDERBAAN, S. O. DUMOULIN;
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Abstract: Introduction Neural responses in early visual cortex are strongly modulated by image contrast. These neural responses do not sum linearly to an increase in contrast, but saturate at high contrast. Contrast response functions (CRFs) are typically measured using synthetic images that are systematically manipulated. Ultimately, these measures are assumed to extrapolate to natural images. Here we measure the fMRI responses in early visual cortex to natural images. We use the inherent variation in contrast in natural images to derive the CRF. Methods We measured fMRI responses elicited by different types of visual stimuli using a 7T MRI scanner. First, we estimated the region of visual space a voxel responds to: the population receptive field (pRF, Dumoulin et al, 2008). The stimuli consisted of conventional bar-shaped population receptive field mapping stimuli with natural image content. We used a 2D Gaussian to represent the pRF. Convolution of the visual field mapping stimulus with the pRF gives a prediction of the fMRI time-series. The pRF parameters were estimated by minimizing the residual errors of the predicted and measured fMRI time-series. Second, we measured the response amplitude elicited by viewing natural images (45 natural images and 1 full-contrast synthetic image). The natural images were taken from the 'Berkeley Segmentation Dataset and Benchmark' database (Martin et al, 2001). Third, we computed the RMS contrast of the natural image within each pRF. We derived the contrast response function by plotting the fMRI response amplitude against the RMS-contrast of all pRFs within a given visual field map. Results The pRF estimates using natural image content were comparable to those previously published with synthetic image content. Typically, we recorded high fMRI response amplitudes to the viewing of natural images. However, these amplitudes varied across the cortex even for single image presentations. When plotting these response amplitudes against RMS contrast, we derived the CRF. This CRF does not sum linearly with increasing contrast, but shows the characteristic non-monotonic increase in response amplitude with RMS contrast. Discussion We show that it is possible to derive the CRF in early visual cortex without systematically varying the contrast. We use the pRF model and contrast variations inherent to natural images. The CRF is comparable to those derived from synthetic image manipulations. These CRFs can be modulated by different mechanisms. This new method provides us with a tool to test hypotheses of effects on the CRF, to give us more understanding of the underlying mechanisms of the contrast response.

Disclosures: W. Zuiderbaan: None. **S.O. Dumoulin:** None.

Poster

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Support: NIMH R01-MH092345 (JTS)

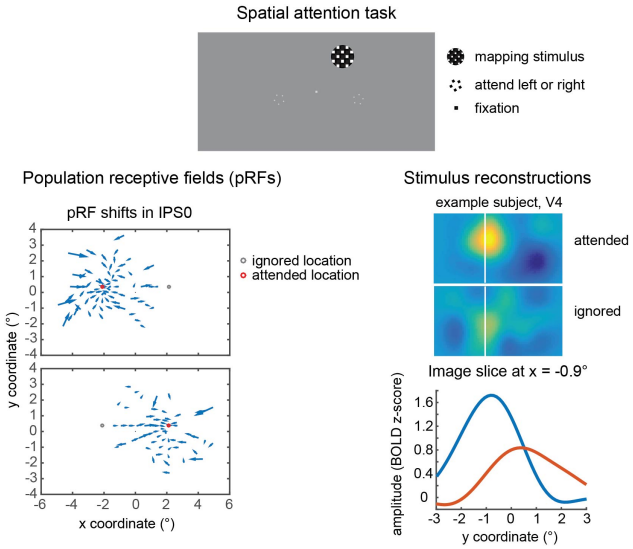
NSF GRFP (VAV)

NSF GRFP (TCS)

Title: Linking attentional modulations of single-voxel population receptive fields and region-level spatial reconstructions

Authors: *V. A. VO, T. C. SPRAGUE, J. T. SERENCES;
Neurosci. Grad. Program, Psychology, UCSD, La Jolla, CA

Abstract: Studies in macaques and humans have documented significant changes in visual receptive field properties with covert attention shifts (Womelsdorf et al., 2006; Klein et al., 2014; Sheremata & Silver, 2015). It is hypothesized that these changes improve the fidelity of visual representations at the attended location (reviewed in Anton-Erxleben & Carrasco, 2013). This is supported by neuroimaging studies which find that the pattern of changes across population receptive fields (pRFs) jointly improves the fidelity of larger-scale neural representations (Kay et al., 2015; Sprague & Serences, 2013). However, unlike macaque electrophysiology studies, subjects did not maintain a static focus of attention, and instead attended to the mapping stimulus. We sought to quantify: 1) how pRF properties change when spatial attention is directed to a fixed location and 2) how changes in pRF response properties jointly impact the quality of region-level representations in retinotopic visual and posterior parietal cortex. Subjects (N = 5) covertly attended to a fixed location in the left or right hemifield (2.1° eccentricity), or to fixation, while small flickering checkerboards (1.4° diam.) mapped voxel spatial selectivity across the visual field (up to 4.5° ecc). We estimated spatial selectivity profiles for each voxel (pRFs) and then used all voxels in a region to estimate a spatial encoding model. We inverted the encoding model to reconstruct images of the stimulus under different attention conditions. We determined pRF changes by comparing responses during attention to fixation with responses during attention to the left or right. We found that pRFs within 1° of the attention locus increased in amplitude. Many pRFs also decreased in size and shifted toward the attention locus. We then compared stimulus reconstructions across attention conditions. Stimulus reconstructions near the attention locus increased in amplitude. Our results support the hypothesis that collective changes in receptive field properties increase the fidelity of visual representations.



Disclosures: **V.A. Vo:** None. **T.C. Sprague:** None. **J.T. Serences:** None.

Poster

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NIH 1 R01 EY02391501A1

NIH 1 RO1 EY02231801A1

Title: The field of view available to cortical reading circuitry

Authors: ***R. LE**, M. BARNETT, B. WANDELL, N. WITTHOFT;
Stanford Univ., Stanford, CA

Abstract: Background. An important part of skilled reading is the ability to quickly and efficiently see words. The proper spatial and temporal resolution, as well as the appropriate field-of-view, are required for rapid word recognition. If only a small part of the field of view is delivered to the reading circuitry, word recognition will necessarily be slowed. Measurements over the last decade have shown that as children learn to read, circuits in ventral and lateral occipito-temporal cortex rapidly recognize word forms (Wandell, 1999). Here, we use population

receptive field (pRF) measurements (Wandell & Winawer, 2015) to characterize the field of view delivered to cortical reading circuitry in skilled readers. **Methods.** We made functional MRI measurements in eight subjects. Using a localizer that compared words with objects, faces, bodies, scenes, and numbers, we identified the visual word form area (VWFA) in ventral occipital cortex of each subject. We then performed pRF mapping with moving bar stimuli (Dumoulin and Wandell, 2008); the contrast pattern within the bar was either words, checkers, or false fonts. The visual stimuli spanned a range of +/- 16 deg of visual angle. For each voxel in the brain, we fit its pRF: a 2D Gaussian (with a peak of 1) in the visual field. The parameters of the pRF are its center location (x,y) and its spread (σ).The pRF can be thought of as the region in visual space that significantly drives responses in the voxel. We visualize visual field coverage by taking the maximum pRF value at each point in the visual field. **Results.** When word stimuli were used within the bars, fitting the pRF model resulted in over 76% of the voxels having variance explained exceeding 10%. Of the voxels exceeding this 10% threshold, the mean variance explained was 32%. (In V1, the mean variance explained of voxels exceeding the 10% threshold is 49%). The visual field coverage of the VWFA extends to about 10 deg. The sizes of the pRF spread are larger in VWFA than at corresponding eccentricities in V1 or V2. While we could not identify a retinotopic map, the visual field coverage was reliable in each subject. **Conclusions.** It is possible to assess the visual field coverage within cortical circuitry essential for skilled reading. In English readers this cortical region responds to stimuli spanning about 10 deg of visual angle, and the region is most responsive to stimuli along the horizontal meridian. **References.** Wandell, B. A., Rauschecker, A. M., & Yeatman, J. D. (2012). Learning to see words. *Annual review of psychology*, *63*, 31. Wandell, B. A. (1999). Computational neuroimaging of human visual cortex.*Annual review of neuroscience*, *22*(1), 145-173.

Disclosures: R. Le: None. **M. Barnett:** None. **B. Wandell:** None. **N. Witthoft:** None.

Poster

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NIH T32-MH20002-15 (TCS)

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HHMI Predoctoral Fellowship (SI)

James S McDonnel Foundation Scholar Award (JTS)

Title: Different population-level measurements and analysis techniques enable complementary insights into attentional modulation of visual responses

Authors: *T. C. SPRAGUE[1], S. ITTHIPURIPAT[1], J. T. SERENCES[1,2];
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Abstract: Visual attention enables protection of relevant information representations from degradation (Sprague, et al, 2015). When contrast response functions (CRFs) are measured across spatial attention conditions using different measurement tools, several modulation patterns are observed: contrast gain (the CRF shifts leftward), response gain (response increases multiplicatively with attention), and additive shifts (response amplitude increases by a constant amount across all contrasts). When measured using fMRI, attention results in a purely additive increase in CRFs (Buracas & Boynton, 2007; Murray, 2008; Pestilli et al, 2011). However, CRFs based on stimulus-evoked responses measured using EEG exhibit either response gain or contrast gain (Kim et al, 2007; Lauritzen et al, 2010; Itthipuripat et al, 2014a; 2014b). We sought to directly compare attentional modulation of CRFs quantified using several analysis techniques applied to fMRI and EEG data acquired while the same participants performed identical tasks with controlled behavioral performance. Participants (n=7) viewed a display featuring a flickering checkerboard stimulus presented at 1 of 6 contrasts. On half of trials, they attended to the checkerboard; on the other half, they attended to the fixation point. During fMRI scanning, on separate runs participants viewed a spatial mapping stimulus consisting of checkerboards presented at different locations across the screen so we could estimate voxel-level population receptive fields (pRFs) and an inverted encoding model (IEM; Sprague & Serences, 2013) used for stimulus reconstruction. In fMRI measurements, we exclusively observed additive shifts: univariate activation levels, stimulus reconstruction amplitude, and activation levels in voxels sorted by pRF position and size relative to attended position all showed additive shifts (in V1-hV4). However, we found evidence for both response gain and additive shifts in EEG measurements: response gain was observed in early ERP components (P1/N1 complex and late positive deflection, LPD) and steady-state visually-evoked potentials (SSVEPs); while additive shifts were observed in alpha band desynchronization and the late sustained contralateral negative ERP waveform. While there are differences in fMRI measurements and stimulus evoked responses measured by EEG, similar results seen between fMRI measurements and the late negative ERP component and alpha desynchronization suggest a potentially similar source for these modulations. Finally, these data place constraints on mechanisms of attentional modulation, which must account for all simultaneous modulations that are observed.

Disclosures: **T.C. Sprague:** None. **S. Itthipuripat:** None. **J.T. Serences:** None.

Poster

700. Organization of ExtraStriate Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 700.07/P2

Topic: D.04. Vision

Support: Nicolás Gravel was supported by the (Chilean) National Commission for Scientific and Technological Research (BECAS CHILE).

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Title: Changes in the temporal dynamics of BOLD co-fluctuations underlie the variability of cortico-cortical population receptive field maps derived from resting-state data

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Abstract: In previous work, we have shown that population cortico-cortical receptive field maps can be derived from resting state (RS) functional magnetic resonance imaging (fMRI) applying connective field (CF) modeling (Gravel et al., 2014). This model-based analysis estimates the spatial integration between blood-oxygen level dependent (BOLD) signals in distinct cortical visual field maps using fMRI. Just as population receptive field (pRF) mapping predicts the collective neural activity in a voxel as a function of response selectivity to stimulus position in visual space, CF modeling predicts the activity of voxels in one visual area as a function of the aggregate activity in voxels in another visual area. In combination with pRF mapping, CF locations on the cortical surface can be interpreted in visual space, thus enabling reconstruction of visuotopic maps from resting state data. Hence, once we know the layout of V1's visual field map, CF modeling of RS activity allows us to derive extrastriate visual field maps resembling those derived by conventional methods. Remarkably, estimates of CF properties differ slightly when estimated with different RS scans, even in the same scanning session. This indicates that - despite uncertainty from methodological origins and considerable inter-scan variability- blood-oxygen level dependent (BOLD) co-fluctuations in the early visual cortex preserve fine-grained visuotopic organization, even in the absence of visual stimulation. Here, we ask which changes in the BOLD signal affect the variability of RS-CF estimates, and, more generally, how estimates of RS-fMRI connectivity in the early visual cortex change over time. We find that the

resemblance between VFM and RS derived extrastriate CF maps is partly explained by a similar periodic structure in the BOLD activity. In particular, the presence of low frequency (~0.01-0.1 Hz), phase-locked BOLD co-fluctuations within the RS visual system seems to underlie the resemblance between connective field maps obtained from RS and VFM data. During VFM, the BOLD responses are locked to strong position-varying visual stimuli. As during RS scans there are no changes in visual stimulation, we speculate that the VFM-like periodic structure in the BOLD signals may be caused by changes in the endogenous state of the early visual cortex.

Disclosures: N. Gravel: None. **B. Harvey:** None. **S. Dumoulin:** None. **B. Curcic-Blake:** None. **F. Cornelissen:** None.

Poster

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Title: Lines of Baillarger *in vivo* and *ex vivo*: myelin contrast across lamina at 7T MRI and histology

Authors: *A. FRACASSO[1], S. J. VAN VELUW[2], F. VISSER[3,4], J. J. M. ZWANENBURG[3], S. O. DUMOULIN[5], N. PETRIDOU[3];
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Abstract: Introduction Myelin concentrations vary across the cortex and cortical lamina. These variations in myelin concentrations are the basis for the parcellation of human cortex. Recent advances in *in vivo* MRI allow the visualization of myelination contrast and have in particular focused on the stria of Gennari in primary visual cortex. Here we focus on the stria of Gennari as well as laminar contrast outside primary visual cortex and the occipital lobe with the aim to reveal the lines of Baillarger, in-vivo. Methods We extended a 3D T1-weighted MPRAGE (T1-w) sequence to optimize contrast between low and high myelin content within human cortex for

ex-vivo and in-vivo imaging. Images were acquired at 7 Tesla (Philips, NL) with a volume transmit and 32-channel receive head coil (Nova Medical, USA). We measured laminar profiles within the human cortex comparing ex-vivo, histological measurements and in-vivo data. Histology: striate and extra-striate areas were sampled and 4μm-thick sections were stained with Luxol Fast Blue-Periodic Acid Schiff for myelin. Ex-vivo MR imaging parameters: TE: 7.7/3.5ms, flip: 8 deg, resolution 400 μm isotropic, 80 slices, TI:280ms, Time delay (TD): 2s. In-vivo MR imaging parameters (4 adult participants): TD: 6s,TI: 1200ms, TR/TE 8/3ms, flip angle: 8 deg, voxel size = 500 μm isotropic. 60 coronal slices, bandwidth 202Hz/px, turbo factor: 275, and adiabatic inversion. Laminar profiles: MRI samples were manually segmented and laminar profiles were extracted from 0.36mm diameter cylinders, from the gray/white matter border to the gray surface. 100 profiles were extracted for each region. Laminar profiles from each area were derived from 1000 bootstrapped datasets. Results *Ex vivo* T1-w laminar profiles showed a consistent T1-w intensity increase for both calcarine and extra-calcarine areas of the cortex, located in the middle of the cortical thickness. These laminar profiles are comparable to those found on the histological sections. Comparable profiles were also obtained on in-vivo T1-w images in healthy adults on calcarine and extra-calcarine areas. Here the laminar profile identifying a T1-w increase in the middle of the cortical thickness extended into parietal and frontal lobes. Conclusion We reliably identify a distinct myelin variation in the middle of the cortical thickness that extends beyond primary visual cortex in ex-vivo and in-vivo images. We propose that this structure represents the lines of Baillarger. These findings provide the basis to apply the principles of myeloarchitectonics, *in vivo*, in humans, using high field MRI.

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Poster

700. Organization of ExtraStriate Cortex

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Topic: D.04. Vision

Support: NIH grant R01 EY017081

Title: Columnar segregation of color- and disparity-selective stripes in human areas V2 and V3

Authors: *R. B. TOOTELL[1], J. R. POLIMENI[2], S. NASR[2];
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Abstract: Background: In non-human primates (NHPs), secondary visual cortex (V2) is made up of repeating columnar 'stripes', labeled with cytochrome oxidase (CO) histology. Among these, the CO-dark 'thin' and 'thick' stripes respond selectively to variations in stimulus color and binocular disparity, respectively. Hypotheses: The NHP data, plus human histology, predict that any homologous color- and disparity-selective stripes in humans should: a) radiate 'outwards' from the V1-V2 border, b) be spaced ~7 mm apart (full cycle), c) be non-overlapping and interdigitated, and d) have segregated functional connections. Additionally, e) stripes for disparity (but not color) should terminate a few mm from the V1-V2 border, and f) similar columns might exist in adjacent area V3. Approach: We used specialized fMRI techniques (7T, 0.8–1 mm iso) to reveal V2 stripes in 6 human subjects. Color-selective stripes were revealed using color- vs. luminance-varying gratings. Disparity-selective stripes were localized using random dot stereograms, presented at near-through-far vs. zero disparity. Areas V2 and V3 were localized retinotopically. A cortical surface analysis enabled extensive signal averaging across multiple scan sessions (thus increasing the contrast/noise), plus selective sampling of activity from the lower cortical layers (minimizing contributions from pial veins). Results: All predictions were confirmed. Color- and disparity-selective stripes radiated perpendicularly to V1/V2 border, spaced 7.2 mm apart. The stripe maps correlated strongly across sessions (e.g. $p < 10^{-30}$). Interdigitation of color- and disparity-selective stripes was confirmed as percent overlap when the maps were topographically aligned, vs. spatially shuffled. An analogous functional segregation was found in V3. This visualization of multiple types of columns is novel in human extrastriate cortex, especially in V3. Next we measured functional connections in the resting state (eyes closed) in both functional types, in V2 and V3. In a double dissociation, activity fluctuations in functionally alike stripes (e.g. color-to-color) correlated more strongly than in unlike stripes (e.g. color-to-disparity), within and between V2 and V3. Conclusions: Prior fMRI results showed differences between specific human visual cortical areas. Here we found a more detailed segregation of feature-selective streams within areas V2 and V3, which extends streams that are known to link V1 and V2 in NHPs. This color- vs. disparity-selective segregation may be a special case of a broader parallel analysis of visual surfaces vs. boundaries, respectively.

Disclosures: R.B. Tootell: None. **J.R. Polimeni:** None. **S. Nasr:** None.

Poster

700. Organization of ExtraStriate Cortex

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Topic: D.04. Vision

Support: NIH intramural research

Title: Curvature-biased cortical areas in human visual cortex and its functional implications

Authors: *X. YUE[1], A. GANDHI[2], L. G. UNGERLEIDER[2];
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Abstract: In a recent fMRI study, we observed a network of cortical areas selective for curvature processing in macaque visual cortex. These curvature-biased areas partially overlapped with the well-studied face-processing areas. Here, we aimed to determine whether human homologues of monkey curvature-biased cortical areas exist and, if so, their anatomical relationship to the face-processing cortical areas in the human brain. FMRI was acquired in a 7T scanner in response to the visual presentation of round vs. rectilinear shapes matched to those used in the monkey fMRI study. Our results revealed significant fMRI biases in the human ventral visual pathway to round vs. rectilinear shapes. Areas biased to round shapes included human V4, OFA, FFA1, and part of V8. A curvature-biased area in human V4 corresponded to the posterior curvature-biased patch in macaques. The curvature-biased areas in OFA and FFA1 appeared to be the homologues of the middle curvature-biased patch in macaques. However, it remains unclear from our data where the human homologue of the monkey anterior curvature-biased patch is, or if it exists. The close proximity of curvature-biased and face-processing areas in both species indicates a functional link between face and curvature processing, which we investigated in two psychophysical experiments using humans. In the first experiment, a sample image (a computer generated face or chair) was presented briefly on each trial, followed immediately by a mask (curved or straight) to interrupt visual processing. The mask was then followed by a 2-choice recognition test. The presentation time of the sample images varied from trial to trial using a staircase method to make performance comparable across conditions. Our results showed that subjects required significantly longer viewing time of the sample images to perform the face recognition task with curved masks compared to straight masks ($t(15) = 3.25$, $p < 0.01$). This pattern was reversed for the chair recognition task ($t(15) = -2.34$, $p < 0.05$). To address the concern that the above pattern of results could be explained by the smooth surfaces in computer generated faces rather than the irregular features found in real faces, we conducted a second experiment using images of natural faces and scenes. As before, these images were presented briefly before and after a curved or straight mask. Subjects categorized faces as male/female, and scenes as forest/non-forest. Thus far, our data suggest that curved masks caused a higher error rate than straight masks in the face task, while the reverse true in the scene task. These findings support the idea that curvature and face processing are functionally related.

Disclosures: X. Yue: None. **A. Gandhi:** None. **L.G. Ungerleider:** None.

Poster

700. Organization of ExtraStriate Cortex

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Topic: D.04. Vision

Support: ICORE (RM)

HBP (RM)

Kimmel award (RM)

US-Israel Binational Science Foundation (LYD)

Title: Why can't we see our own eyeblinks? an ECoG study

Authors: T. GOLAN[1], I. DAVIDESCO[3], M. MESHULAM[4], D. M. GROPPE[5], P. MÉGEVAND[5], M. S. GOLDFINGER[5], E. YEAGLE[5], L. MELLONI[6], C. E. SCHROEDER[6,7], A. D. MEHTA[5], L. Y. DEOUELL[1,2], *R. MALACH[4];
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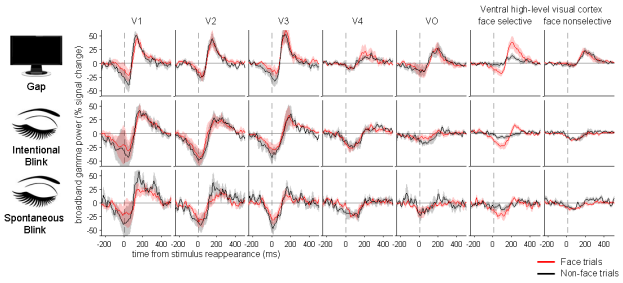
Abstract: The perceived continuity of the visual image despite frequent interruptions by eye blinks is a ubiquitous visual illusion. We investigated the neural correlates of this illusion using electrocorticography in patients being evaluated for epilepsy surgery. Patients were presented with blocks of still images while monitored for spontaneous and intentional eye blinks. In control blocks, the retinal impact of these events was mimicked by artificial darkenings of the screen ('gaps'). We found that in V1, the disappearance of the visual stimulus due to either gaps or blinks led to a drop in broadband gamma activity. The reappearance of the stimulus was followed by a rebound of the activity beyond the pre-drop levels. Surprisingly, high-level ventral visual areas such as the Fusiform-Face Area exhibited a post-disappearance drop that was slightly more pronounced for blinks than for gaps, an effect inconsistent with the intuitive account for blink invisibility by perceptual 'filling-in'. In contrast, the rebound elicited by the termination of gaps and blinks indeed reflected the established sensation-perception gradient across the ventral visual stream hierarchy: when moving downstream from the early visual cortex towards high-level ventral visual cortex, the rebound response subsided for blinks more than for gaps. This pattern was observed both within single patients and at the group level (the accompanying figure depicts offset-locked grand averages). In conclusion, broadband gamma

response in ventral visual areas can indeed be viewed as a correlate of the perceptual distinction between blinks and external darkenings. However, the temporal structure of this response does not directly correspond with the apparent continuity of the perceived visual image.



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Poster

700. Organization of ExtraStriate Cortex

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Topic: D.04. Vision

Support: NIH Grant MH93567

Title: A shift toward dendrite targeting inhibition in extrastriate visual cortex

Authors: *D. MORROW-JONES[1], A. DISNEY[2];
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Abstract: The primate neocortex has distinct populations of inhibitory interneurons that have been classified using various anatomical and functional criteria. One such classification scheme is based on immunoreactivity for the calcium-binding proteins parvalbumin (PV), calbindin (CB), and calretinin (CR). The PB-, CB-, and CR-immunoreactive populations are believed to represent non-overlapping classes that together make up ~95% of all inhibitory interneurons in macaque V1. Furthermore, these subpopulations have some correspondence with classically-defined morphological neuron types and with physiological phenotypes. The cell body laminar distribution of these neuron subpopulations has been previously characterized in the primary visual cortex (V1) of macaques. 74% of all inhibitory interneurons in V1 are estimated to be PV-ir. These PV-ir cells are present in all layers except layer I, with a heavy band in layer IVc. CB-ir

neurons, on the other hand, are mainly present in layers II and III (van Brederode et al., 1990), and CR-ir neurons are mainly present in layer II (Meskenaite, 1997). This laminar distribution of cell bodies appears to be conserved in the frontal lobe. The prefrontal cortex (PFC) has PV-ir neurons in all layers besides layer I, especially layers III and IV. Similar to V1, PFC has a strong PV-ir neuron presence in layer IV. In addition, the PFC has CB-ir neurons mostly in layers II and III, and CR-ir neurons mostly in layer II (Condé et al. 1994). However, the PV-ir neuron predominance seen in V1 is not observed in the PFC. Instead, the CR-ir population is estimated to be twice that of CB-ir or PV-ir neurons. In macaque V4, we characterized the distribution of calcium-binding protein expressing neuron subpopulations using triple label immunofluorescence confocal microscopy. Labeled neurons were counted for each layer. Similar to V1 and PFC, V4 PV-ir neurons are present in all layers besides layer I, especially layers II/III and IV. In addition, CB-ir neurons are mostly present in layers II and III, and CR-ir neurons are mostly present in layer II. PV-ir neurons are the predominant subpopulation, similar to V1, but comprise only half the population of calcium-binding protein expressing neurons. There was virtually no dual or triple labeling for any class. PV-ir neurons often target the soma or axon, in contrast with prominent dendritic targeting seen in CB-ir and CR-ir neurons. This allows PV-ir neurons to regulate an integrated response in pyramidal cells. Our findings suggest an increased emphasis of dendritic integration as a method of inhibitory signaling in downstream cortical regions, as a result of a relative decrease in the PV-ir neuron population.

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Poster

700. Organization of ExtraStriate Cortex

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Program#/Poster#: 700.13/P8

Topic: D.04. Vision

Support: NSERC

OGS

Title: Age-related shift in E-I balance in dorsal visual pathway

Authors: *C. SIU[1], K. M. MURPHY[2];
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Abstract: Many aspects of visual perception decline in older adults, but surprisingly they perform better than young adults on motion discrimination that activates surround suppression. An age-related loss of GABA-mediated surround suppression is the main hypothesis to explain that better motion discrimination. However, there is global age-related loss of GABA in the cortex but not global changes in visual tasks that rely on surround suppression. Alternatively, local changes in other neurotransmitters systems might contribute to the loss of surround suppression. For example, in visual cortex feedforward-driven activity is strongly mediated by AMPA receptors, while feedback modulation is mediated by NMDA receptors. Perhaps, expression of these glutamatergic receptors is maintained in the dorsal/motion visual pathway but lost in other visual areas. To address this question we used Western blotting to quantify expression of AMPA (GluA2) and NMDA (GluN1) receptor subunits in V1, V3 (dorsal), and V4 (ventral) of human postmortem tissue samples. We studied 15 cases ranging in age from 5-80 years. We also measured the expression of GAD65, the enzyme that makes the on-demand pool of GABA. First, we compared expression of GAD65 in V1, V3, and V4 of human cortex. We found similar age-related losses of GAD65 in all 3 areas. Next we compared expression of GluA2 and GluN1 among the 3 areas. We found similar losses in V1 and V4, but surprisingly no loss of these glutamatergic receptor subunits in V3. Furthermore, only the dorsal pathway had a change in GAD65 vs GluA2 and GluN1 that could shift the excitatory-inhibitory balance in favor of excitation. These findings provide new information about pathway specific neurobiological changes in the aging human visual system. The maintenance of high levels of glutamate receptors in the dorsal pathway suggests that this excitatory neurotransmitter system is likely to have a greater contribution to motion perception in aging. Finally, the findings point to the E-I balance rather than absolute levels of excitation or inhibition as important for surround suppression in aging visual perception.

Disclosures: **C. Siu:** None. **K.M. Murphy:** None.

Poster

701. Binocular Vision: Stereopis and Amblyopia

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

Support: NEI EY013644

Title: Perception of depth sign from motion parallax relies on combining extra-retinal signals regarding eye and body rotation

Authors: *V. KOGAN[1], D. E. ANGELAKI[2], G. C. DEANGELIS[1];
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Abstract: Motion Parallax (MP) refers to the relative image motion of objects located at different depths, caused by the translation of the observer. In the absence of pictorial depth cues, MP is ambiguous regarding depth sign (near vs. far). Retinal motion of a near object during a leftward translation could be the same as retinal motion of a far object during rightward translation. Psychophysical studies (e.g., Nawrot & Joyce 2006) have shown that smooth eye movements can disambiguate depth sign from MP. Theoretical work by Nawrot and Stroyan (2009) showed that the critical variable for disambiguating depth sign from MP is the rate of change of eye rotation relative to the scene. Importantly, eye rotation relative to the scene (R_{es}) is determined by the combination of eye rotation relative to the head (R_{eh}), head rotation relative to the body (R_{hb}), and body rotation relative to the world (R_{bw}): $R_{es} = R_{eh} + R_{hb} + R_{bw}$. Thus, the brain should ideally combine the relevant extra-retinal signals and use the resultant (R_{es}) to disambiguate depth sign from MP. Alternatively, the brain might rely on smooth pursuit signals (R_{eh}) as an approximate solution. To explore this issue, we trained macaques to discriminate depth sign from MP, manipulating depth coherence to control task difficulty (Kim et al. 2015). The animal was translated back and forth by a motion platform (to generate MP), and was required to execute smooth eye movements to maintain fixation on a world-fixed target. In addition, the motion platform was used to rotate the body around an axis through the eye. Thus, R_{es} was determined by the combination of passive body rotation (R_{bw}) and active eye pursuit (R_{eh}) - there was no rotation of head on body ($R_{hb} = 0$). By presenting various combinations of R_{bw} and R_{eh} that all produced the same R_{es}, we could examine which variables determine perceived depth sign. If perception relies on R_{es}, then all combinations of $R_{bw} + R_{eh}$ should evoke equivalent performance. If perception relies solely on R_{eh}, performance should decline as R_{bw} increases, reaching chance when $R_{bw} = R_{es}$ ($R_{eh} = 0$). When $R_{bw} > R_{es}$, such that the sign of R_{eh} reverses, perceived depth sign may reverse. Results to date show robust depth discrimination performance for a variety of combinations of R_{eh} and R_{bw} in which $R_{bw} \leq R_{es}$ (including $R_{eh} = 0$), indicating that vestibular rotation signals contribute to disambiguating depth sign. However, performance is erratic when R_{eh} and R_{bw} have opposite signs ($R_{bw} > R_{es}$, $R_{eh} < 0$), suggesting that depth perception may not rely on a true representation of R_{es}. This paradigm will provide an excellent vehicle for exploring how different sources of rotation signals contribute to neural computations of depth from MP.

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Poster

701. Binocular Vision: Stereopis and Amblyopia

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Program#/Poster#: 701.02/P10

Topic: D.04. Vision

Support: ERC parietal action

Title: The role of disparity and viewpoint in the processing of observed actions

Authors: *S. FERRI, G. RIZZOLATTI, G. A. ORBAN;
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Abstract: The present human fMRI study investigated the role of disparity and the observer's point of view in processing of observed manipulative actions. The actions (grasping, dragging, pushing, displacing) were simultaneously recorded by two camcorders supplied by 3D lenses, one on the left of the actor and the other facing him. 21 right-handed subjects underwent to two fMRI sessions (16 runs), where 2D or 3D action videos were contrasted in a 2x2x2 block design: viewpoint (frontal/lateral), spatial dimensions (2D/3D), video-type (action/control videos: static and dynamic-scramble) with separate runs for the two controls. To control both saccades and vergence, we used a binocular eyetracker confirming steady fixation. In the first level analysis motion in depth was taken as a variable of not interest, because dynamic disparity differed between experimental and scrambled videos. The three-way interaction, computed at the second level and conjoined for the two types of control conditions, positive for frontal view, 3D and actions, produced three sites in the left hemisphere: DIPSM (-22 -56 54 t=5.6; FWE p=0.05), PFop (-54 -28 20 t=5.7; FWE p=0.006) and PreCentral Gyrus (PrCG, -40 -4 48 t= 5.9 FWE p=0.002). Splitting the data according to viewpoint indicated that dimension and video-type interacted only for frontal and not lateral viewpoint. We noticed that the motion in space of three out of four action exemplars was congruent with the frontal viewpoint. Hence the interaction sites were either specific for frontal viewpoint or for action along the Z-axis of the observer. We therefore ran a control experiment with viewpoint and direction in space as factors. ROI analysis on 15 subjects showed the activity in all regions was stronger for actions out of the fronto-parallel plane of the observer (2way ANOVA interaction: DIPSM $p<0.01$; pfOP: $p<0.005$; PrCG: $p<0.005$). However the profiles of the four conditions were different in the three ROIs: 3 (area) x4 (conditions) ANOVA (interaction $p<0.001$). The main effect of view point was significant only in PrCG ($p<0.05$). Thus stereo enhances action processing in parietal and premotor regions, an effect strongest for out of the fronto-parallel plane actions. Viewpoint enhanced the stereo effect only at the premotor level. This study is supported from ERC Grant

Disclosures: S. Ferri: None. **G. Rizzolatti:** None. **G.A. Orban:** None.

Poster

701. Binocular Vision: Stereopis and Amblyopia

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Support: NSF Research Grant BCS-1354029

Title: Natural binocular fixations in humans and binocular disparity

Authors: *M. S. BANKS[1], W. W. SPRAGUE[2], S. RESSIER[1];
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Abstract: The two views of the world from forward-facing eyes in humans and other species generate binocular disparities from which precise depth estimates can be derived. There is keen interest in the distributions of disparities that occur naturally in different parts of the visual field. To determine those distributions, one must know the 3D geometries of natural scenes and where viewers look in those scenes. Several investigators have estimated disparity distributions from simulated scenes and eye fixations. Others have measured distance distributions in real scenes and then produced simulated fixations in those scenes. Recently, we measured binocular eye position and 3D scene geometry simultaneously as humans performed natural tasks in a variety of scenes (Sprague et al., 2015). From those measurements, we determined the natural distributions of disparities across the central visual field. The distributions are consistent with disparity preferences among V1 neurons of primate. The distributions of binocular fixation are not consistent with those from previous simulations because viewers are biased toward fixating nearer points among all visible points and are biased toward looking up and down and left and right rather than in all directions equally. We examined how disparity distributions depend on observed binocular fixations. We did this by computing mutual information between vergence eye position (convergent vs divergent) and disparity, and between version eye position (left-right, up-down, etc.) and disparity. Mutual information is a measure of variables' mutual dependence with few assumptions about the type of dependence (unlike the correlation coefficient). We found that mutual information is high between vergence and horizontal disparity in the upper and lower visual field, but not the left and right fields. We also observed that mutual information is very high between vergence and vertical disparity for oblique regions of the visual field. We observed clear dependency between horizontal version and vertical disparity and between version and horizontal disparity. Our results show quite clearly that knowing binocular eye position and 3D scene geometry simultaneously is essential to determine the disparities human normally encounter.

Disclosures: **M.S. Banks:** None. **W.W. Sprague:** None. **S. Ressier:** None.

Poster

701. Binocular Vision: Stereopis and Amblyopia

Location: Hall A

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Title: Effects of drift speed and stimulus size on binocular rivalry rate and mixed percept duration in healthy individuals: Implications for endophenotype studies in clinical psychiatric groups

Authors: *S. MILLER[1,2], P. C. F. LAW[1,2], T. T. NGO[1,2,3];
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Abstract: Presenting conflicting images, one to each eye, results in perceptual alternations known as binocular rivalry (BR). BR rate (BRR) is slower in the heritable psychiatric condition, bipolar disorder (BD) compared with healthy controls (HC). BRR is also around 50% genetically determined. Thus slow BRR rate has been proposed as an endophenotype for BD. To assess this endophenotype potential, large-scale studies are required with sample sizes in the thousands to tens of thousands. In preparation for such work, we have been examining two issues: the relationship between individuals' eye movements and their BRR (reported in Law, Paton, Riddiford, Gurvich, Ngo, and Miller, in press), and the stimulus parameters that maximize separation between BD and HC. Here we report the effects of stimulus drift speed (a measure of stimulus strength) and size on BRR in HC. We previously reported that gratings drifting at 4 cycles/sec better separated BD and HC than stationary gratings, perhaps due to the different stimulus strengths' effect on HC. In the present study, we varied stimulus drift speed to assess whether BRR can be further increased in HC. We also assessed mixed percept duration (MPD) with each drift speed, aiming to minimize MPD. In addition, we assessed the effect of varying stimulus size on BRR and MPD. We conducted a repeated-measures within-subjects study of HC (N=40, mean age=34.4) viewing BR on a True3DiTM liquid-crystal display monitor through passive linear polarizer 'filters' at a distance of 3 m. Stimuli were green, orthogonally-drifting vertical and horizontal square-wave gratings with spatial frequency 8 cycles/deg (contrast=0.99;

mean luminance=4.8 cd/m2) in a circular aperture on a black background. Drift speed and aperture size varied across four conditions: (i) 4 cycles/sec at 1.5° and (ii) 8 cycles/sec at 0.5°, 1° and 1.5°. BRR was stabilized for 7 minutes, followed by four counterbalanced 7-min test blocks. We found: (i) for 1.5° stimuli, 8 cycles/sec produced faster BRR (median=0.52Hz, s.d.=0.24) than 4 cycles/sec (median=0.47Hz, s.d.=0.22) ($p<0.01$), (ii) BRR was slower for 8 cycles/sec in a 0.5° aperture (median=0.40 Hz, s.d.=0.16) compared with all other stimulus conditions ($p<0.001$), (iii) MPD was shorter for a 1.5° aperture than a 1° aperture at 8 cycles/sec ($p<0.01$), and (iv) MPD was longer for a 0.5° aperture compared with all other stimulus conditions ($p<0.001$). The findings suggest that stimuli drifting at 8 cycles/sec in a 1.5° aperture could maximize group separation in endophenotype studies of BRR, without introducing higher MPD. However, we are currently repeating these experiments with BD subjects before instigating this protocol change.

Disclosures: **S. Miller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SMM is co-inventor on a University of Queensland patent. There are currently no commercial activities.. **P.C.F. Law:** None. **T.T. Ngo:** None.

Poster

701. Binocular Vision: Stereopis and Amblyopia

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Topic: D.04. Vision

Support: CIHR Grant 74700

FRQS Grant 29695

Title: Loss of binocular depth perception by genetic functional inactivation of ipsilateral retinal projections

Authors: ***T. DOLIQUE**[1], S. SEDIGHI[2], K. CULLEN[2], F. CHARRON[1,3,4,5,6];
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Abstract: Binocular vision allows depth perception through the convergence of visual information from both eyes in the brain. During development, most retinal ganglion cell (RGC) axons cross the midline at the optic chiasm and project toward the opposite side of the brain (contralateral axons), while a small number of axons remain on the same side (ipsilateral axons). Ipsilateral projections have been suggested - with no formal proof as yet - to be important for depth perception. Here we used a genetic approach to functionally inactivate ipsilateral projections and investigate whether these projections are important for depth perception. Synaptic transmission was suppressed in ipsilateral-projecting RGCs (iRGCs) by conditionally expressing the tetanus toxin light chain subunit (which inhibits neurosecretion) using a Cre-mediated recombination approach. Interestingly, we showed that the functional inactivation of iRGCs did not induce changes in the retinal patterning and did not prevent their axons to maintain their normal trajectory, target to the appropriate brain regions, segregate and consolidate their normal amount of target territory into the late postnatal period. Most importantly, using visual tests, we demonstrated that while these transgenic mice maintain proper visual discrimination, they fail to detect depth in a binocular visual test. Therefore, our data provide for the first time a clear demonstration of the specific requirement of functional ipsilateral retinal projections for binocular depth perception.

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Poster

701. Binocular Vision: Stereopis and Amblyopia

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Topic: D.04. Vision

Support: NWO VICI

ERC

Title: A probabilistic approach to multiple object tracking in three-dimensions

Authors: ***J. R. COOKE**[1], R. J. VAN BEERS[2,1], A. C. TER HORST[1], P. MEDENDORP[1]; [1]Donders Ctr. For Cognition, Radboud Univ. Nijmegen, Nijmegen, Netherlands; [2]Fac. of Human Movement Sci., VU Univ. Amsterdam, Amsterdam, Netherlands

Abstract: Many daily situations require us to track multiple objects and people, from avoiding walking into people in the street to monitoring the traffic around us as we drive. Our multiple

object tracking (MOT) ability has traditionally been investigated in stationary observers tracking objects in a plane. This simplifies the task and does not address how observers track objects when targets move in 3D and when the observers move themselves. Having objects move in depth adds additional complexity to tracking. Depth estimation requires integration of complex cues such as disparity, relative size, shading and motion parallax. These cues also depend on our self-motion, for example motion parallax is a sum of parallax from self-motion and object motion. As such to effectively track in depth the brain must integrate as well as disambiguate cues. Here, we are studying how stationary observers track objects in 3D. A prominent approach to studying how observers solve a task given the available cues is to create a Bayesian ideal observer model and compare its performance to human performance. We have developed a Bayesian multiple object tracking model which performs MOT by estimating the time-varying state (position and velocity) of objects using noisy observations provided from cues such as retinal motion, disparity, relative size and motion parallax. To perform this state estimation it is important that sensory measurements are assigned to the correct object, the data association problem. This is crucial in MOT as objects move close to each other and so generate sensory measurements that could be from either object. Our model treats this data association probabilistically using a particle filter that samples possible associations. Combined this provides a model of how a Bayesian observer tracks objects taking into account sensory uncertainty. Simulations of this model show that adding, removing or altering sensory cues will alter tracking ability. One prediction is that observers tracking objects moving at a given speed will be more accurate when the objects move in depth (with disparity, motion parallax and size as cues) than if objects move at the same speed in a plane. We are currently testing this prediction using a 3-D display which renders moving objects in 3-D using disparity, motion parallax, relative size and shading cues. Our results confirm the prediction that tracking with multiple depth cues improves tracking compared to tracking in a plane.

Disclosures: **J.R. Cooke:** None. **R.J. Van Beers:** None. **A.C. Ter Horst:** None. **P. Medendorp:** None.

Poster

701. Binocular Vision: Stereopis and Amblyopia

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Topic: D.04. Vision

Support: NIH Intramural Research Program

Title: Disparity selective adaptation to correlated and anticorrelated patterns in V1 neurons requires computation across a broad population of neurons

Authors: *P. L. APARICIO, B. G. CUMMING;
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Abstract: Adaptation is ubiquitous in the visual system. Here we describe the effect of adapting to both correlated (cRDS) and anti-correlated random dot stereograms (aRDS) on 42 disparity selective V1 neurons recorded from two fixating macaque monkeys. The aRDS elicit disparity selective responses in many V1 neurons that are inverted relative to the responses produced by cRDS. As reported in other domains, adaption frequently produced changes in response gain, which we estimated using linear regression of adapted on unadapted responses. The slope defined a response gain ratio produced by adaptation. We also computed a "preferred index" quantifying whether the adapting disparity was closer to the neuron's preferred or null disparities. For disparities close to the preferred disparity, adaptation to cRDS produced response decreases (mean gain ratio 78%, n = 28), while adaptation to aRDS produced response increases (mean gain ratio 113%). Disparities closer to the null disparity produced the opposite pattern (mean ratio 110% for cRDS, 88% for aRDS, n = 36). The slope of the relationship between preferred index and gain ratio had similar magnitudes but opposite signs for cRDS (-0.42) and aRDS (0.37) Thus, cRDS and aRDS had opposite effects on the responsiveness of disparity selective neurons that were approximately equal in magnitude. This is surprising as aRDS typically produce weaker responses. We also observed clear adaptation to aRDS in neurons that showed no disparity selective responses to aRDS. To examine this in the population, we used a median split on the response modulation to aRDS. Adaptation to aRDS produced similar effects in the two groups: the slope relating "preferred index" to response gain was 0.33 in cells strongly modulated by aRDS, and 0.32 in the weakly modulated group. Many neurons also showed stimulus specific adaptation effects, not captured by changes in gain/offset of the whole response curve. Preferred cRDS produced a stimulus-specific response reduction (mean 21%), that declined as a Gaussian function of distance from the adaptor (SD 0.07 degrees). The same disparities in aRDS produced the opposite effect, smaller in magnitude (mean 7%). Thus, adaptation to disparity in cRDS produced effects in V1 neurons that are comparable to the effects previously reported for other stimulus dimensions. Adapting to aRDS produces equivalent, but reversed effects. The magnitude of this effect, however, was not related to the strength of responses to aRDS in individual neurons. This implies that adaptation is not driven simply by the relationship of the responses of a single neuron to the adapting stimulus, but depends on the properties of a broad population of neurons.

Disclosures: P.L. Aparicio: None. **B.G. Cumming:** None.

Poster

701. Binocular Vision: Stereopis and Amblyopia

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James S. McDonnell Foundation

Title: Binocular imbalance in macaque MT in strabismic amblyopia

Authors: *T. J. VAN GROOTEL, J. A. MOVSHON, L. KIORPES;
New York University, Ctr. For Neural Sci., New York, NY

Abstract: Cortical area MT plays a central role in processing information about motion and depth. V1 cells and some MT cells sense the motion of image components, while other MT cells signal the motion of integrated patterns - a property known as pattern direction selectivity (PDS). Most MT cells in normally reared animals receive input from both eyes and respond similarly to stimulation through either eye, a key component of their sensitivity to stereoscopic depth. Both of these properties are immature in infant macaques, suggesting that their development might be vulnerable to abnormal postnatal visual experience. Strabismus, a misalignment of the visual axes, often leads to amblyopia, a disorder in which visual acuity is reduced in one eye and stereopsis is often abolished. Some amblyopic observers also have difficulty detecting visual motion with their amblyopic eye, especially for stimuli of slow and moderate speed. To investigate the neural correlates of the motion processing deficits in strabismic amblyopia, we have recorded from neurons in MT of an awake, fixating strabismic amblyopic macaque monkey. We used drifting gratings, plaids, and dot fields to measure direction selectivity, speed tuning, spatiotemporal tuning and eye dominance in amblyopic MT cells. Most MT neurons in this animal were monocular, and only about ¼ were dominated by the amblyopic eye. The tuning properties of cells driven by the amblyopic eye were similar to fellow eye cells. PDS cells were equally prevalent among cells dominated by the two eyes, and, for binocularly driven cells, tuning properties were the same regardless of viewing eye. However, in a small population of cells tested binocularly as well as monocularly, we did not see any change in firing rate during binocular viewing compared to viewing through the fellow eye alone, suggesting that the fellow eye suppressed activity evoked by the amblyopic eye when both were stimulated. Reflecting the overall shift in eye dominance, firing rates were lower when viewing with the amblyopic eye. The receptive field positions of MT neurons were not shifted proportionally to the misalignment of the eyes. We conclude that MT neurons in amblyopic monkeys have similar response properties in the two eyes, but that neurons driven by the amblyopic eye are weakly responsive

and numerically underrepresented. The lack of influence of the amblyopic eye under binocular viewing conditions may reflect an active process that filters out the input from the amblyopic eye.

Disclosures: T.J. Van Grootel: None. **J.A. Movshon:** None. **L. Kiorpes:** None.

Poster

701. Binocular Vision: Stereopis and Amblyopia

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Topic: D.04. Vision

Support: NIH Grants EY05864

Title: Radial frequency discrimination is impaired in amblyopic non-human primates

Authors: *S. SHARIAT TORBAGHAN, B. N. BUSHNELL, L. KIORPES;
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Abstract: Radial frequency (RF) stimuli have been used to assess global shape discrimination in human subjects. RF patterns are circular patterns in which the base circle is sinusoidally modulated; they are described by the frequency and amplitude of that modulation. The ability of human subjects to discriminate the modulation is in the hyperacuity range at threshold - finer than 1 arc min. It has been hypothesized that these stimuli modulate the responses of V4 neurons in extrastriate visual cortex, where neurons are thought to encode curvature and global form. Amblyopic human adults and children have previously been shown to perform poorly on RF discriminations, suggesting that there may be a deficit in processing these hyperacuity-range stimuli at the level of V4. Amblyopia is a disorder of spatial vision that results from abnormal visual experience in early life. Amblyopia has been associated with a wide range of spatial visual deficits, including poor performance on Vernier acuity tasks which are also typically performed in the hyperacuity range. To assess whether performance on RF shape discriminations reflects a similar deficit to Vernier acuity, and in preparation for electrophysiological recording studies in V4, we assessed the performance of macaque monkey subjects on RF and Vernier acuity tasks. We trained 7 non-human primates (*Macaca nemestrina*) ranging in age from 33 to 630 weeks, 4 strabismic amblyopic and 3 visually-normal controls, to perform 2AFC discrimination tasks. For the RF task, animals were shown two circular patterns, one with and one without radial deformation. They indicated which stimulus contained the deformations as a function of amplitude and frequency of the modulation. The youngest animal showed higher RF

threshold than older controls and fellow eyes of amblyopes, especially at the higher RF values. Amblyopic animals' performance was impaired with their amblyopic eye compared to both their fellow eye and control animals, especially at the higher RF values. We also tested Vernier acuity (VA) in a subset of amblyopic animals and compared their losses on the two tasks. We found that the RF deficits at high RF values were larger than the VA deficits, suggesting that they do not depend on the same underlying mechanisms. The RF deficits may therefore represent a specific disorder of global shape perception which may reflect disorder of extrastriate visual processing.

Disclosures: S. Shariat Torbaghan: None. **B.N. Bushnell:** None. **L. Kiorpes:** None.

Poster

701. Binocular Vision: Stereopis and Amblyopia

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Topic: D.04. Vision

Support: NIH Grant EY008128

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NIH Grant EY007551

Title: Receptive-field center/surround interactions in V2 neurons of amblyopic monkeys

Authors: *B. ZHANG[1], X. TAO[2], E. L. I. SMITH[2], J. WENSVEEN[2], Y. CHINO[2];
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Abstract: Beyond the well-established loss of contrast sensitivity and acuity, amblyopic primates exhibit a variety of complex spatial vision deficits; in particular, amblyopic children and adults exhibit position uncertainty, image distortion, and poor contour integration. What is common in these perceptual impairments is their inability to accurately encode, weigh, and pool local stimulus feature information over the extended range of space. We previously found that the receptive-field (RF) subunit maps of V2 neurons in amblyopic monkeys show substantial 'disarray' (heterogeneity), which may explain some of the perceptual impairments in behavioral tasks that require orderly integration of visual features over space. In this study we examined the RF center surround interactions of V2 neurons to explore the wider range of spatial interactions covered by individual neurons in amblyopic monkeys. We found that the strength of surround suppression over the center responses was abnormally elevated in V2 neurons of amblyopic

monkeys. Moreover, the strength of surround suppression paralleled the degree of disruptions in RF subunit maps (higher average heterogeneity index values) for individual amblyopic monkeys. When we stimulated the small, restricted regions of RF surround, using grating patches that are placed across the unit's RF center for 4 different positions (i.e., top-bottom, side, oblique 45/225, and oblique 135/315), we observed surround facilitation depending on the locations of surround patches in both normal and amblyopic monkeys. In normal monkeys, the highest surround facilitation occurred for the collinear surround patches (top-bottom) as previously reported. However, in those neurons driven by the amblyopic eye showed the weakest surround facilitation with co-linear surround patches. These results suggest that the long-range intrinsic and/or feedback connections in V2 are abnormal in amblyopic monkeys, and that this neuronal abnormality may be related to their poor performance in contour integration tasks.

Disclosures: B. Zhang: None. **X. Tao:** None. **E.L.I. Smith:** None. **J. Wensveen:** None. **Y. Chino:** None.

Poster

701. Binocular Vision: Stereopis and Amblyopia

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Support: PEW Scholars 26-7522-1050

CRCNS 26-1679-2050

Title: Loss of binocular disparity selectivity following monocular deprivation in mouse V1

Authors: *J. PATTADKAL, B. SCHOLL, N. PRIEBE;
Inst. for Neurosci., The Univ. of Texas at Austin, Austin, TX

Abstract: Experience dependent plasticity during the critical period of development shapes anatomical and functional elements of cortical circuits. In primary visual cortex (V1) of mice, neurons in binocular zone shift preference toward the ipsilateral eye if the contralateral eye is occluded during the critical period. This shift equalizes the relative contribution of input from each eye. Here we tested how this increased ocular input affects binocular disparity selectivity of V1 neurons, a response property arising from the integration of ocular inputs. Using two-photon calcium imaging we measured ocular dominance (OD) and disparity selectivity of neurons in the binocular zone of mice after occluding one eye during the critical period. Surprisingly, a

decrease in disparity sensitivity accompanied increased binocularity in deprived animals. The median ODI changed from -0.36 to -0.03 in monocularly deprived animals while the DSI was 0.2 in normal (n = 878 cells) and 0.14 in monocularly deprived animals (n = 752 cells). Decreased disparity tuning was most pronounced in moderately binocular neurons. The decline in disparity could reflect the formation of disparity selectivity during the critical period or a disruption of disparity selectivity by deprivation. To distinguish between these possibilities, we measured disparity selectivity at the onset of the critical period and found similar ranges of disparity selectivity as in adult animals (median DSI = 0.21, median ODI = -0.38, n = 916 cells). Therefore the decline in disparity selectivity by monocular deprivation reflects a disruption of selectivity by deprivation and not an interruption in the emergence of disparity signals. Our results indicate that changes in ocular dominance by monocular deprivation are not solely due to changes in the synaptic weights of existing connections, but also the emergence of novel synaptic connections, which could not be guided by disparity signals during monocular deprivation. Therapies to improve binocular function often include the occlusion of one eye, which increases the degree to which cortical neurons are sensitive to the open eye, but may also disrupt binocular integration in visual cortex.

Disclosures: **J. Pattadkal:** None. **B. Scholl:** None. **N. Priebe:** None.

Poster

702. Eye Movements and Perception

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 702.01/P20

Topic: D.05. Visual Sensory-motor Processing

Title: Dynamic representation of saccades in mouse frontal cortex

Authors: *R. KIMURA[1], H. OSAKI, M[1], T. K. SATO, M[2], T. R. SATO, M[1,3];
[1]Werner Reichardt Ctr. for Integrative Neurosci., Tuebingen Univ., Tübingen, Germany; [2]UCL Inst. of Ophthalmology, London, United Kingdom; [3]JST, PRESTO, Tuebingen, Germany

Abstract: Saccades brings the eyes toward an object of interests. These voluntary eye movements are essential in information processing, and observed in various species including mice. While the circuit-level questions in mice have been addressed effectively, the cortical circuits underlying saccades has remained unapproachable, due to the lack of behavioral paradigm for mouse saccades. Here using a newly developed behavioral paradigm in mouse saccades, we show the dynamic nature of the mouse cortical circuits that controls the two eyes with narrow overlap of the visual field. The movements of the two eyes were coupled during the

voluntary saccades, and these binocularly coupled saccades were controlled by a small region of the medial prefrontal cortex (mPFCeye). Microstimulation of mPFCeye induced binocularly coupled contraversive saccades. Optogenetic inhibition of mPFCeye suppressed contraversive but not ipsiversive saccades. *In vivo* two-photon calcium imaging revealed greater activity of neurons in this region during contraversive saccades. However, the neural circuitry responsible for the contraversive saccades is not hard-wired but more adaptable. mPFCeye acquired the capacity to control ipsiversive saccades within days of contralateral mPFCeye optogenetic inhibition. Therefore, saccades are dynamically regulated by cortical circuits, allowing mice to adjust their visuo-motor behavior in ever-changing environment through binocularly coupled eye movements.

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Poster

702. Eye Movements and Perception

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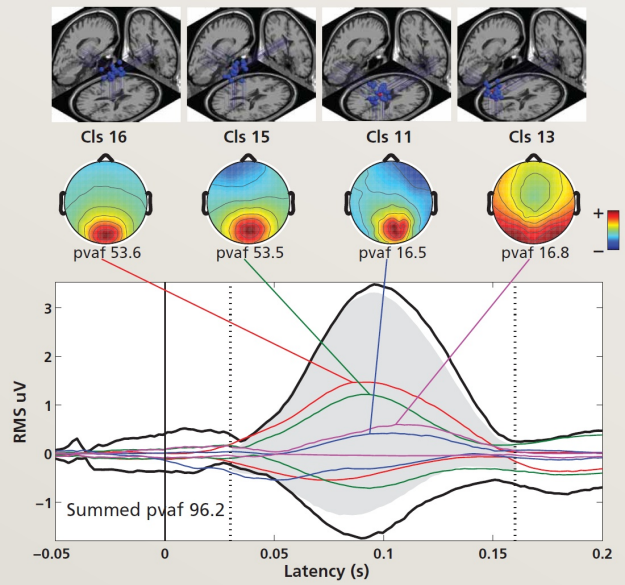
Topic: D.05. Visual Sensory-motor Processing

Title: Decomposing saccade-related potentials using ICA

Authors: ***M. MIYAKOSHI**[1], O. BALKAN[1], C. LEE[1], F. MEDEIROS[2], S. MAKEIG[1]; [1]Swartz Ctr. For Computat. Neuroscience, INC, UCSD, La Jolla, CA; [2]Dept. of Ophthalmology, Univ. of California San Diego, La Jolla, CA

Abstract: We developed a novel active visual search task in which participants saccade freely across an evolving display of Gabor patches. Here we report event-related potentials (ERPs) time-locked to saccade events identified using independent component analysis (ICA). Participants were 20 healthy young adults (10 female, mean 20.9 yrs, SD 3.0, range 18-26). Five Gabor patches were always present on a gray background. A sequence of 1200 patches were pseudo-randomly selected from four patch sizes and five orientations (SOA 2 sec, duration 5 sec). The task was to detect infrequent (15%) instances in which the size and orientation of the newest patch matched those of another patch that was still visible, prompting a button press. Eye-tracking data were obtained from the left eye while 205-channel, 512-Hz scalp EEG data were recorded. Around 350/4000 independent components of EEG were grouped into 19 clusters with the criterion of anatomical locations of ICs. The results confirmed that the total of 80 ICs included in the four occipito-patietal clusters account 96.2 % of variance in the lambda response, a classical occipital ERP component presumably phase-locked to fixation onset, observed in the

scalp channel measurement. In the figure, black traces represent the envelopes, maximal and minimal channel values, of the lambda response time locked to fixation onset; colored traces represent the envelopes of the mean projections of independent component processes in 4 posterior IC clusters. The results suggest that the lambda response has a distributed network over the occipito-parietal regions, and they are activated in different time courses. We conclude that ICA-based ERP analysis on saccade could shed light on the distributed network across the occipito-parietal lobes.



Disclosures: **M. Miyakoshi:** None. **O. Balkan:** None. **C. Lee:** None. **F. Medeiros:** F. Consulting Fees (e.g., advisory boards); Allergan Inc, Carl Zeiss Meditec Inc, Novartis. Other; Alcon Laboratories Inc, Bausch & Lomb, Carl Zeiss Meditec Inc, Heidelberg Engineering Inc, Merck Inc, National Eye Institute, Reichert Inc, Sensimed, Topcon Inc. **S. Makeig:** None.

Poster

702. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

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Title: Saccadic preparation at the presentation of visual stimuli to the leading and unleading eye

Authors: *V. MOISEEVA[1,2], M. SLAVUTSKAYA[1], V. SHULGOVSKIY[1];
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Abstract: When oculomotor system selects a stimulus as a saccadic target there is a simultaneous inhibition of other possible responses, irrelevant for the current behavior. The cognitive processes of attention and decision-making play an active role in the saccade programming. The aim of our research was to analyse spatial-temporal parameters of saccades and presaccadic ERP-potentials at the simultaneous presentation of the target and distracting stimuli to the leading and unleading eye. 15 healthy right-handed volunteers participated in the study. Target and distracting peripheral visual stimuli were presented monocularly on the monitor in various spatial combinations. Eye movements were recorded using the EOG. The LP of correct saccades increased when the target and distracting stimuli were presented in the different visual hemifields at the distance 15-20 dergees and were minimal when the stimuli were presented at the same hemifield at the distance 5 degrees. At the same time the number of erroneous saccades was the biggest. More often error was a saccade to the distracting stimulus the first and to the target one the next. Such incorrect saccades appeared when LP saccades decreased by 50-60 ms compared to the LP of correct saccades ($p < 0.05$). The complex of the positive and negative potentials was revealed in the saccade latent period. Latency of all components was shorter upon presentation of stimuli to the left, unleading eye, that may indicate the earlier saccade preparation. At the same time LP saccades were longer in this conditions ($p<0.05$). The results show that early potentials N1 and P1 were higer in amplitude and dominated in the controlateral parietal-occipital areas. It can be reflection of visual sensory processing. The amplitude of the later negative potential N2 at the stimulation of the right eye increased in the case when target stimulus was at the same location than at the previous realisation. At the backward averaging of EEG from the saccade beginning premotor potentials have been obtained and analyzed. Potential N-1 immediately preceding the beginning of saccade and associated with activation of neurons in the contralateral oculomotor fields in the parietal cortex (LIP and 7a). This study found the fact of changing the localization of N-1 potential. It had a maximum amplitude and dominated in the frontal eye fields contralateral to the hemifield where the distracting stimulus were located. Such localization of N-1 foci may be associated with an active inhibition of leading oculomotor fields of frontal cortex in case of avoid the saccade initiation to irrelevant stimulus.

Disclosures: V. Moiseeva: None. **M. Slavutskaya:** None. **V. Shulgovskiy:** None.

Poster

702. Eye Movements and Perception

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 702.04/P23

Topic: D.05. Visual Sensory-motor Processing

Title: Power and frequency of human visually induced gamma-band activity reflects retinal but not perceived stimulus speed

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Abstract: Human visually induced gamma-band activity is modulated by cognitive factors like attention and by stimulus factors like e.g. contrast and motion speed. Increasing stimulus speed leads to increasing gamma power and frequency. Interestingly, very similar stimulus motion on the retina can also be generated by movements of the eyes, i.e. smooth pursuit. The self-generated retinal motion is however not perceived as external stimulus motion (except for minute illusory effects). This offers the possibility to investigate whether gamma reflects retinal motion or rather the perceived motion. We recorded MEG from 15 human subjects, while they saw visual grating stimuli that were either static or moved at one of two speeds, and while they held their eyes still or moved them at one of two speeds. This allowed us to disentangle whether gamma depended on retinal speed or on perceived speed. We find that gamma power and gamma frequency over visual cortex are positively correlated with retinal speed, irrespective of whether retinal movement was due to stimulus movements or eye movements. Gamma power and frequency were not correlated to perceived speed, when changes in perceived speed did not entail changes in retinal speed, i.e. when the eyes moved with the stimulus, thereby holding the stimulus stable on the retina, but giving the perception of a moving stimulus. We also investigated alpha power and found a different pattern. Alpha power was negatively correlated with pursuit speed, irrespective of whether changes in pursuit speed led to changes in retinal speed or perceived speed. With the current paradigm, it was not possible to exclude that this effect is related to increased attentional demands introduced by pursuing a moving target compared to fixation. Alpha power was not correlated with changes in retinal or perceived speed, if they were not accompanied by changes in pursuit speed. Our results suggest that the dominant alpha and gamma MEG sources are differentially related to retinal and eye speed. This is relevant for the concept of predictive coding, which suggests that top-down predictions are subtracted from sensory evidence and only the resulting prediction error is sent in the bottom-up direction. In our experiment, gamma depended on retinal speed in a similar way, when retinal speed changed due to external factors, or due to internal and thus predictable factors. This

suggests that in this case, gamma reflects the sensory evidence rather than the prediction error. Visually induced gamma in human MEG recordings is likely dominated by primary visual cortex. Further analyses at the source level will determine whether alpha and gamma show differential patterns across cortex.

Disclosures: **B.F. Handel:** None. **P. Fries:** None.

Poster

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Topic: D.05. Visual Sensory-motor Processing

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Title: A coarse-to-fine neural dynamics resulting from eye movements

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Abstract: Multiple studies support the notion that the human visual system follows a coarse-to-fine dynamics, in which the gist of the scene is examined before fine details. The mechanisms responsible for this dynamics remain unclear. On the basis of neurophysiological recordings in cats and monkeys, it has been hypothesized, that neurons in the early visual system undergo similar dynamic changes in their response sensitivity, progressively shifting their receptive field tuning to higher spatial frequencies during the course of a fixation. However, the processes mediating such a fast reshaping of the neurons' receptive fields have never been identified. Here, we provide a novel account that suggests that coarse-to-fine processing of visual input might be a consequence of oculo-motor behavior. Under natural viewing conditions, humans continually alternate large saccades with periods of small fixational movements. These movements yield sequential temporal transients in the visual signals impinging on the retina, which appear compatible with a coarse-to-fine dynamics. To examine the potential contributions of eye

movements, we examined the time-course of neural activity during natural post-saccadic fixation in standard models of retinal ganglion cells. These models enable analytical expressions of the temporal evolution of the neuronal frequency sensitivity. Our theoretical analysis shows that immediately following a saccade, cell responses are dominated by the low spatial frequency components of the visual input. However, shortly after fixation onset, sensitivity starts to gradually shift toward higher spatial frequencies, so that, approximately 150ms after fixation onset, neurons no longer respond to low spatial frequencies. By means of computer simulations, we show that this dynamics of neural activity can be easily mistaken for a change in the cell receptive field during the course of fixation, even though the simulated cell filter does not change. The apparent changes in receptive fields peak frequency and size in our simulations were very similar to those reported in neurophysiological experiments but were caused by the dynamic reorganization of power across spatial frequencies operated by the natural alternation between saccades and fixational eye movements. Our results carry important implications for the encoding of visual information. They suggest a scheme of neural encoding in which eye movement transients play a critical role and visual information is timed to fixation onset. In this scheme, a coarse-to-fine processing of the visual input is not an intrinsic processing strategy of the visual system but a consequence of oculo-motor behavior.

Disclosures: **A. Casile:** None. **M. Rucci:** None.

Poster

702. Eye Movements and Perception

Location: Hall A

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Program#/Poster#: 702.06/P25

Topic: D.05. Visual Sensory-motor Processing

Title: Head centered encoding in monkey area V1 as the basis of a veridical percept of upright despite false torsion of the eyes due to Listing´s law

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Abstract: Goal-directed saccades and the fixations that follow are governed by Listing´s law that restricts all rotations necessary to move the eyes from any starting position to new positions to a plane that is specific to that starting position. According to Listing´s law the eyes rotate about the line of sight, a “false” torsion that grows with eccentricity when the eye is at an oblique (or “tertiary”) position. Although false torsion causes considerable image tilt (e.g. 9° for an

oblique eccentricity of 30°/30°), we do not perceive a tilted image. As first shown by Hermann von Helmholtz in his "Handbuch der Physiologischen Optik" (1867) this reinterpretation of the tilted image tilt as being upright requires the consideration of non-visual information on the eye movement. Although known to be necessary for more than 140 years, it has remained completely unclear how the integration of nonvisual information ensuring the maintenance of a veridical percept of upright is implemented in our visual system. We hypothesized that the perceptual compensation of false torsion is an achievement of primary visual cortex (V1) as we had previously shown that a significant subset of neurons in primary visual V1 encodes space relative to the head rather than the eye if body tilt causes ocular counterroll (Daddaoua et al. Nat. Com. 2014). To test this hypothesis we carefully characterized the receptive fields (RFs) of V1 cells of rhesus monkeys during fixation at 3 different gaze positions: straight ahead, 20°/20° upper right and -20°/20° upper left with the visual display always oriented tangentially to the line of sight. Eye position was documented by a combination of 2D-search coil recordings and an analysis of eye rotation about the a-p eye axis using high resolution video images. The latter demonstrated that the difference in false torsion between the two opposite oblique position amounted to around 9° as predicted by Listing´s law. Our preliminary analysis of the receptive fields of 52 neurons mostly recorded from supragranular layers shows that indeed many of them encode spatial positions in a head centered frame of reference, i.e. they shift their position on the retina taking information on the amount of false torsion into account.

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Poster

702. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

Title: Contributions of response magnitude and variability to the presaccadic enhancement of visual representations

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Abstract: Modulation of the presaccadic activity of extrastriate neurons has been suggested to underlie the representational enhancement of saccadic targets. In order to understand how changes in firing rate, correlated activity (noise correlation) and response variability (Fano factor) of extrastriate neurons contribute to presaccadic representational enhancement, we used 16 channel linear array electrodes to simultaneously record the activity of multiple neurons in the middle temporal (MT) cortex of macaque monkey. Two monkeys were trained to maintain fixation while a target stimulus was presented for 1s within the neurons' receptive field (RF). At the end of the trial, animals moved their eyes either toward or away from the stimulus, as instructed by a visual cue. Similar to previous studies of presaccadic responses in V4, we found an increase in firing rate and a reduction in Fano factor in the responses of MT neurons prior to saccades toward a target within their RF. Moreover, we found that the correlation in trial-to-trial variability between pairs of MT neurons drops prior to a saccade into their RF. The increased magnitude and reduced variability of presaccadic responses resulted in an enhanced representation of visual targets, as quantified by the performance of a support vector machine classifier designed to discriminate the orientation of the RF stimulus based on neuronal responses. In order to understand the relative contributions of response magnitude and variability to this representational enhancement, we developed an artificial spike generator to control for the effects of rate as well as correlated and independent variability (noise correlation and Fano factor, respectively). Our results indicate that changes in the response magnitude play a prominent role in driving the presaccadic enhancement of visual representations. These findings contrast with previous reports that changes in correlated variability, rather than firing rate, are the primary drivers of representational enhancement during covert attention, potentially indicating different mechanisms of representational enhancement during covert and overt attentional deployment.

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Poster

702. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

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Title: Visual and presaccadic activity in Area 8A of the dorsolateral prefrontal cortex in macaque monkeys

Authors: *K. BULLOCK[1,2], F. PIEPER[3], A. SACHS[4], J. C. MARTINEZ-TRUJILLO[5]; [1]Western Univ., London, ON, Canada; [2]Dept. of Physiol., McGill Univ., Montreal, QC, Canada; [3]Univ. Med. Ctr. Hamburg-Eppendorf (UKE), Inst. for Neuro- & Pathophysiology, Hamburg, Germany; [4]Dept. of Surgery, The Ottowa Hosp. Res. Institute, Univ. of Ottowa, Ottowa, ON, Canada; [5]Dept. of Physiol. & Pharmacol., Robarts Res. Institute, Western Univ., London, ON, Canada

Abstract: The prefrontal cortex, particularly the dorsolateral area 8A, is thought to play a role in transforming visual signals into goal-directed behavior. Neurons within this area exhibit visual and saccade-related responses during oculomotor tasks. Several trends in spatial encoding are observed in visual and oculomotor-related areas, including the frontal eye fields, the primary visual area, and the superior colliculus. For example, the receptive fields and movement-related fields in these areas are retinotopically organized, exhibit a gaussian shape, and scale with eccentricity. It is unclear whether receptive fields and movement-related fields in area 8A share these characteristics. Here we recoded the responses of 166 neurons in area 8A of two male Macaca fascicularis monkeys while the animals made visually-guided saccades to a peripheral sine-wave grating stimulus positioned at one of 40 possible locations (8 angles arranged along 5 eccentricities). To characterize the receptive fields and movement-related fields, we fit a bivariate gaussian model to the average firing rate at each location during stimulus presentation (early and late visual epoch) and prior to saccade onset (presaccadic epoch). 40 percent of neurons showed visual and presaccadic responses. Of the spatially-selective neurons, 93 neurons had contralateral-preferring and 25 had ipsilateral-preferring visual receptive fields. We determined that the endpoints of saccades were less accurate at greater target eccentricities ($p<0.05$,ANOVA). Furthermore, we observed that the angular width of visual and movement-related fields scaled positively with increasing eccentricity ($p<0.05$,ANOVA). Finally, we found that most neurons preserve the same directional preference across eccentricity.

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Poster

702. Eye Movements and Perception

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.05. Visual Sensory-motor Processing

Title: Saccadic corollary discharge underlies stable visual perception

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Abstract: Each saccadic eye movement directs the high-resolution foveae of our retinas towards objects of interest. The retinal input therefore changes substantially with each saccade. Our visual perception, however, remains stable despite the massive disruptions in visual input occurring several times per second. Philosophers and scientists over centuries have proposed that our perceived stability of the visual scene depends upon registering out retinal input to an internal signal representing each eye movement. This signal, referred to as corollary discharge (CD), has recently been identified in the monkey brain, but its contribution to visual perception is still a matter of dispute. We now show that disrupting the CD signal in the medial dorsal thalamus by muscimol inactivation alters visual perception. After saccades, we were able to change the monkey's perception of where its gaze was directed. We conclude that the CD is the most critical component for stable visual perception, rather than proprioception or visual cues. Our results show that the same brain area that generates saccades, which cause displacement of the retinal image, also generates the CD signal that allows us to organize jumbled retinal inputs into a stable visual scene.

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Poster

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Topic: D.05. Visual Sensory-motor Processing

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Title: Evidence for an eye-centered perception of stimulus orientation during saccades

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Abstract: Despite the nearly constant motion of information on our retina due to eye movements, we perceive the world around us as stable. Many have investigated how the brain accomplishes this spatial reconstruction during horizontal and vertical retinal displacements, but, surprisingly, little focus has been placed on the effects of torsional rotations. Due to the spherical shape of the eyes, retinal input is rotated during oblique eye orientations. Importantly, Listing's law provides that there is no actual torsion of the eyeballs at these orientations, so, to reconstruct a stable perception of space during eye movements, the brain must utilize an eye orientation-based internal model of this oblique gaze-induced effect. To investigate if this is the case, we induced rotations of retinal input using oblique eye orientations, and presented oriented bar stimuli for one frame (8 ms) pre-, peri- and post-saccadically during horizontal saccades (presentation window from 400 ms before saccade onset to 400 ms after saccade offset). Participants performed 40 deg saccades either along the screen's horizontal meridian (control condition) or along the same line from a vertically eccentric orientation (+20 deg; test condition), producing approximately 2 deg of retinal rotation at maximum eccentricity. Participants were then asked to judge the direction of stimulus rotation relative to vertical in a two-alternative, forced choice task. On average, psychometric PSEs revealed that participants systematically perceived vertically eccentric stimuli on the left third of the screen as rotated by -1.7 deg (average predicted retinal rotation of -2.3 deg), in the middle of the screen as rotated by +0.5 deg (average predicted retinal rotation +0.8 deg), and on the right side of the screen as rotated by +1.6 deg (average predicted retinal rotation +2.0 deg). In comparison, control trials along the horizontal meridian yielded much smaller shifts in PSEs (left: +0.7 deg, middle: +1.1 deg, right: +1.2 deg), suggesting an eye-centered coding of orientation during saccades. Furthermore, for both control and test conditions a shift in the psychometric function began approximately 100 ms prior to saccade onset until about 100 ms after saccade onset, possibly indicating a pre-saccadic remapping effect consistent with previous work on spatial localization. Together, these findings suggest that during saccades in the absence of surrounding visual cues, perceptual processes rely

more heavily on retinal inputs than on extraretinal inputs that are ultimately required for a stable perception of the world.

Disclosures: **T. Murdison:** None. **G. Blohm:** None. **F. Bremmer:** None.

Poster

702. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

Support: NIA AG031769

Title: High-gain visual feedback impairs response time in older adults

Authors: ***M. KWON**, Y.-T. CHEN, E. A. CHRISTOU;
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Abstract: Older adults exhibit slower response time due to longer premotor time. Premotor time has been associated with visual information processing. It remains unknown whether and how a task with greater visual information processing requirements will influence response time in older adults. The purpose of this study, therefore, was to determine the effect of visual information processing on response time in older adults and the underlying activation of the motor neuron pool. Participants conducted a submaximal constant force task (15 % of maximum) with ankle dorsiflexion for 20 s and dorsiflexed their ankle as fast as possible in response to a visual stimulus. The visual information processing was manipulated by changing the amount of force visual feedback into a high-gain (1.2°) or low-gain (0.5°) condition. There are three novel findings from this study. 1) High-gain visual feedback slows the response time in older adults because it lengthens pre-motor time. 2) High-gain visual feedback further slows response time in older adults because it increases force variability. 3) The slower response in older adults with high-gain visual feedback is related to altered modulation of the motor neuron pool. Specifically, older adults exhibited less oscillations within 4-10 Hz and greater oscillations from 10-35 Hz with high-gain visual feedback. This findings provide novel evidence that high-gain visual feedback contributes to slower response times in older adults. The slower response time is likely mediated by impaired visual information processing and altered modulation of the motor neuron pool.

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Poster

702. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

Title: Magnifying the scale of visual biofeedback improves posture

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Abstract: Biofeedback (BF) has been evidenced to minimize body sway during quiet standing situations as well as cognitively demanding situations. However, limited research has reported the optimal sensitivity parameters of visual BF related to the center of pressure (COP) sway. Therefore, the main purpose of this study was to further investigate the scale of sensitivity required for optimal visual BF. Accordingly, 19 young adults (6 males; 13 females; aged 21.3 ± 2.5) stood with feet together on a force platform for 60 seconds and performed three visual BF intensities (BF magnified by 1 (BF1), BF magnified by 5 (BF5), BF magnified by 10 (BF10)), along with control trials with no BF (NBF). For example, the gain was set such that 1 cm of COP movement corresponded to 1 cm of movement on the visual BF display for the BF1 condition. The visual BF was displayed on a computer monitor placed 1 meter in front of the participant. During the BF conditions, participants were asked to keep a dynamic rectangle with an area of 2 mm by 2 mm within the limits of a static, 2 mm by 3 mm square which remained in the middle of the screen; the dynamic visual display represented their current COP position. Participants were instructed to stand as still as possible while minimizing the movements of the visual target. The findings revealed that the BF1 scale produced significantly greater COP displacement in both the anterior-posterior (AP) (BF5: $p=0.014$; BF10: $p=0.007$) and medial-lateral (ML) (BF5: $p=0.014$; BF10: $p=0.007$) directions, as well as greater standard deviation (SD) of the COP in the AP direction (BF5: $p =0.012$; BF10 $p=0.013$). Additionally, NBF showed significantly greater 95% Area Ellipse than BF1 ($p <0.001$), BF5 ($p <0.001$), and BF10 ($p <0.001$) intensity. Therefore, the most sensitive COP scales generated the least amount of postural sway. However, there were no significant differences on any of the COP measures between BF5 and BF10. This suggests that the BF5 intensity is sufficient to improve body sway. This research may provide insight with respect to the proper scale on which biofeedback should be given in order to improve postural control. Thus, this study may have implications in both research and rehabilitation settings.

Disclosures: **D.A. Jehu:** None. **J. Thibault:** None. **Y. Lajoie:** None.

Poster

702. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

Support: University of Wisconsin Foundation

The Jane Bradley Pettit Foundation

Title: Enhancement of eye movements in Parkinson's disease as a result of CN-NINM intervention

Authors: *Y. I. VERBNY, G. VISHWANATHAN, K. SKINNER, M. TYLER, K. KACZMAREK, Y. DANILOV;
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Abstract: Objective: The neurorehabilitation of sensory and motor functions in Parkinson's disease (PD) patients is undeveloped and unexplored. There are very few methods that show the possibility of recovery of eye movements affected by PD. The goal of this research was to investigate how well Cranial-Nerve Non-Invasive Neuromodulation (CN-NINM) can reduce the effects of PD-induced impairments of oculomotor function and help to recover eye movement control. Methods: We completed a 4-month intervention with a 66-year-old male 6 years after he was diagnosed with PD-symptoms. The CN-NINM intervention used a combination of both physical and cognitive exercises with electrotactile stimulation to the tongue using a Portable Neuromodulation Stimulator (PoNS). Assessment of oculomotor function was performed before and after the CN-NINM intervention using special 4-channel binocular eye tracking goggles (VisualEyes, Micromedical Inc.). To evaluate the state of subject’s eye movements we used three static nystagmus tests (vertical and horizontal gaze, and spontaneous nystagmus) and three dynamic tests (random saccade, smooth pursuit and optokinetic). All of the tests were performed without tongue stimulation. Results: The CN-NINM intervention resulted in the gradual enhancement of patient eye movement control in all 6 tests. We observed improvement of eye fixation, accuracy and stability in nystagmus and gaze tests, increased eye movement accuracy and precision, improved gain and velocity of target tracking, and changes in both smoothness and synchronization of binocular movement control in oculomotor tests. The most significant improvements in eye movement control were found during performance of smooth pursuit and random saccade testing. Conclusions: Our study establishes a proof of concept and effectiveness of a new non-invasive neuromodulation therapy for rehabilitation of eye movement control. A

combination of exercise and tongue-based neurostimulation may benefit people affected by PD and would offer a novel treatment option for this disease. (No Image Selected) Layman Abstract (optional): Provide a 50-200 word description of your work that non-scientists can understand. Describe the big picture and the implications of your findings, not the study itself and the associated details.: Our study establishes a proof of concept and effectiveness of a new non-invasive neuromodulation therapy. The improvements of eye movement control demonstrated by this individual suggest that rehabilitation using a combination of exercises and tongue-based neurostimulation may benefit people affected by PD and would offer a novel treatment option for this disease.

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Poster

702. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

Title: Temporal coupling of action and perception in Parkinson's disease

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Abstract: Previously, we showed that action and perception are temporally coupled in healthy subjects. Saccadic and perceptual report reaction times (SRT and PRT) share the same time-change dependence upon the temporal asynchrony (TA) between target onset and fixation point offset. We also showed a novel illusion, the misperception of small overlaps as gaps. We hypothesized that the temporal coupling might depend on a prominence map located in the intermediate layer of the superior colliculus (SC). The same mechanism might delay the perception of a target in the presence of competitive stimuli, causing the misperception of briefly overlapping stimuli as separated in time. In Parkinson's disease (PD), increased activity of the substantia nigra pars reticulata induces an excessive inhibition of the SC, which is considered to be responsible for typical eye movement abnormalities. We predicted that increased inhibition of the SC in PD should affect the mechanism that couples action and perception. Six PD patients performed a gap/overlap paradigm. Subjects made saccades to targets with different TA and reported whether they perceived the stimuli as separated by a gap or overlapped in time. SRT, PRT and perceptual transition thresholds were analyzed as functions of TA. SRT and PRT in PD did not show the same time-change dependence upon TA as controls. Although SRT in PD was dependent upon TA, the effect of the SRT dependence (longest minus shortest reaction time) was smaller than in normal subjects. In contrast, PRTs were much longer than normal, with a bigger time-change dependence upon TA. Moreover, PD subjects misperceived small overlaps as gaps for much longer overlapping TAs (< 300 ms) than did controls (< 100ms). Increased basal ganglia inhibition in PD may be responsible for decoupling action and perception and causing a misperception of longer overlaps as gaps than in normal subjects. This action/perception decoupling and the misperception in PD could affect complex functions such as visual search, attentional performances and other tasks that require fine action-perception coordination such as locomotion. A model that explains our results needs greater caudal than rostral inhibition of the SC in PD. This would also explain some typical abnormalities of PD, such as the facilitation of small saccades, staircase saccades, and large square wave jerks. The rostral/caudal activity ratio might be a marker of inhibitory BG output and, therefore, of disease progression.

Disclosures: E. Pretegiani: None. **N. Vanegas-Arroyave:** None. **E.J. FitzGibbon:** None. **M. Hallett:** None. **L.M. Optican:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: BBSRC

Pfizer Neusentis (CASE studentship)

Title: Age-dependent alterations in the excitability of small-diameter IB4-negative mouse dorsal root ganglia neurons

Authors: M. A. MIś[1], E. B. STEVENS[2], *A. D. RANDALL[3,1];
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Abstract: Dorsal root ganglia (DRGs) house the cells bodies of nociceptive afferent neurons, which react to potentially harmful stimuli. Age dependence of nociceptor excitability is an understudied area of pain research and has become particularly relevant with the ageing demographic. The objective of this study was to determine any neurophysiological differences in small-diameter IB4-negative DRG neurons from adolescent, middle-aged and aged male mice. Methods: Dorsal root ganglia cells from 1 month, 8 months and 18 months old male C57/BL6 mice (Charles Rivers, UK) were enzymatically dissociated and cultured for up to 48 hours. Whole-cell voltage- and current-clamp recordings were performed at 35°C. Results: Age-dependent changes in biophysical properties of the small-diameter unlabelled DRG neurons were far less pronounced than the previously reported alterations in the IB4-positive neurons (Miś et al, 2013). There were no significant shifts in the activation or inactivation curves or in the ratio of the tetrodotoxin-sensitive to tetrodotoxin-resistant sodium current. In the current-clamp data, the cells from all three ages were found to have a resting membrane potential of around -55mV. However, input resistance, membrane time constant, sag and firing frequency were all subject to age-dependent changes, with the cells from the middle-aged animals exhibiting the highest levels of excitability. Conclusion: Both passive and spiking properties of mouse small-diameter IB4-negative sensory afferent neurons are age dependent. These results, combined with the previously reported results on IB4-positive neurons, make it feasible to assume that different groups of nociceptors age in disparate ways. References: Miś et al. (2013) Temperature-dependence of tetrodotoxin-sensitive and -resistant Na+ current in IB4+ve neurones from adolescent and adult mouse dorsal root ganglia. 8th Congress of the European Federation of IASP Chapters - PAIN IN EUROPE VIII Florence, Italy, October 9 - 12, 2013.

Disclosures: **M.A. Miś:** None. **E.B. Stevens:** None. **A.D. Randall:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

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Topic: D.08. Pain

Support: NIH AR47410

Title: PSD-95-immunoreactivity in epithelial cells and nerve fibers of the rat cornea

Authors: *B. K. CARR[1], S. A. GEURKINK[2], K. E. MILLER[2];
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Abstract: Background: Upon high threshold stimulation, nociceptive sensory afferents release glutamate from peripheral nerve terminals. Glutamate causes auto- or paracrine activation and sensitization of sensory nerve terminals via excitatory amino acid receptors (EAARs; Miller et al, 2011). Furthermore, glutamate interacts with EAAR's expressed by cells in peripheral tissues, e.g., epithelial cells. In the cornea, immunohistochemical studies have determined that EAAR subunits for NMDA, AMPA, and Kainate (KA) are present on nerve terminals and corneal epithelial cells. Efficient neurotransmitter signaling often relies on scaffold proteins, such as postsynaptic density protein 95 (PSD-95), to anchor postsynaptic receptors and associated proteins. In the current study, we used immunohistochemistry in rat cornea to detect PSD-95 as an initial step in dissecting protein scaffolds used by peripheral nerve fibers and corneal epithelial cells. **Aim:** To determine the distribution of PSD-95 in the rat cornea. **Method:** Sprague Dawley rats were anesthetized and perfused transcardially using fixative. Corneas were collected and corneal cross sections were processed for immunohistochemistry with rabbit antisera for PSD95. Double immunohistochemistry also was performed with anti-PSD and several nerve fiber markers, e.g., PGP 9.5, TRPA1. A Leica confocal microscope was used to evaluate immunolabeling. **Results**: Strong PSD-95 immunoreactivity was observed in the epithelial cell membrane, whereas moderate PSD-95 immunoreactivity occurred in the nerve fibers of the cornea. **Conclusion**: The localization of PSD-95 in the cell membrane of corneal epithelial cells indicates that these cells have a highly organized, intracellular protein scaffold for distribution and localization of extracellular receptors. Exploration of the co-localization of EAAR subunits with PSD-95 and other scaffolding proteins may help to understand the interaction between peripheral afferents and corneal epithelial cells.

Disclosures: B.K. Carr: None. **S.A. Geurkink:** None. **K.E. Miller:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

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RYC2011-08589 (Ministerio de Economia y Competitividad

Title: Investigating the trafficking mechanisms of TRESK, a two-pore domain potassium channel implicated in pain

Authors: *J. P. GIBLIN, D. SUDRIA, A. CASTELLANOS, A. ANDRES, G. CALLEJO, X. GASULL;
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Abstract: The two-pore domain potassium channel TRESK (K2P18.1) has been implicated in pain perception pathways and changes in channel expression and function alter sensory neuron excitability. TRESK has emerged as a therapeutic target with potential use in treatment of pain-related disorders. Despite the importance for overall current density, the mechanisms determining TRESK channel number at the cell surface are not well understood. To investigate factors affecting TRESK trafficking to and from the cell surface we developed a surface biotinylation assay for use in conjunction with electrophysiological analysis. The study described has two objectives (i) to determine the role of the TRESK C-terminus in channel trafficking and (ii) to determine whether TRESK trafficking could be altered by treatment with the Protein Kinase C (PKC) activator Phorbol 12-myristate 13-acetate (PMA). The C-terminus of TRESK contains a conserved tyrosine-based endocytic motif. We hypothesised that this motif is an important determinant of channel endocytosis and that mutation of this motif would increase channel surface expression due to impairment of endocytic internalisation. When surface expression of rat TRESK (rTRESK) and rTRESK with a mutated endocytic motif (rTRESKY389A_V392A) were compared in transfected HEK293 cells by surface biotinylation and patch clamp electrophysiology, significant changes in surface expression and whole-cell currents were not observed. These results suggest that the conserved endocytic motif in the C-terminus of TRESK is not an important determinant of channel expression at the cell surface. Several mediators of inflammation activate Protein kinase C (PKC) dependent signalling pathways. We hypothesised that, as reported for other ion channels expressed in sensory neurons, PKC activation could affect TRESK trafficking. In the study we treated HEK293 cells

transfected with rTRESK with PMA and analysed the effect on the surface expression of TRESK. Treatment with 100nM PMA increased rTRESK surface expression and whole-cell currents. These results raise the possibility that inflammatory mediators may modulate TRESK trafficking, providing a potential mechanism by which sensory neurons can limit hyperexcitability in response to tissue damage.

Disclosures: **J.P. Giblin:** None. **D. Sudria:** None. **A. Castellanos:** None. **A. Andres:** None. **G. Callejo:** None. **X. Gasull:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

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Support: JSPS KAKENHI Grant Number 15K11304

Title: Osmolarity changes contribute to nociceptive sensitization induced by activation of Protease-Activated Receptor 2 (PAR-2) in the rat

Authors: ***K. KIDO**, E. MASAKI;
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Abstract: Background- We previously demonstrated that the activation of proteinase-activated receptor 2 (PAR-2) on afferent nerves caused stronger guarding pain, mechanical and heat hyperalgesia in rat, although the mechanism was unknown. Recently, it has been reported that the activation of PAR-2 causes sensitization of the ion channel transient receptor potential vanilloid 4 (TRPV4) and induces guarding pain and mechanical hyperalgesia. This channel has functions as a transducer of hyper/hypotonic stimuli in primary afferent nociceptive neurons and contributes to inflammatory and neuropathic pain. Therefore, we hypothesized that: (1) incision induces the release of tryptase from mast cell which activates PAR-2; (2) the activation of PAR-2 sensitizes TRPV4; (3) the change of osmolarity in wounds stimulates TRPV4 and increases postoperative pain. In this study, we examined whether the combination of PAR-2 agonist and hyper/hypotonic stimuli increases guarding pain, mechanical and heat hyperalgesia *in vivo* and vitro. Method- The PAR-2-activating peptide, SLIGRL-NH2 was administered by the intraplantar route in the rat with/without hyper/hypotonic solution. Guarding behavior (biting and licking) was observed after administration of SLIGRL-NH2 for 30 minutes. Mechanical thresholds were assessed using von Frey filaments. Withdrawal latencies to heat were assessed

applying a focused radiant heat on the injected area of plantar skin. Using the rat glabrous *in vitro* skin-tibial nerve preparation, afferent activities from single mechanosensitive nociceptors were recorded. Ongoing activities and responses to mechanical stimuli were recorded before and after SLIGRL-NH2 with /without hyper/hypotonic solution application or vehicle. Responses to heat and cold stimuli were recorded once after exposure to either with SLIGRL-NH2 application or control. Results- Hypertonicity and Hypotonicity caused a four-fold increaase in nociceptive behavior by PAR-2 agonist. Also changes of osmolarity increased mechanical and thermal hyperalgesia. *In vitro* skin-nerve preparation, SLIGRL-NH2 with hyper and hypotonic solution increased ongoing activities in 55-60 % C-fibers within 5 minutes. SLIGRL-NH2 application also enhanced the responses to mechanical and heat stimuli in C- fibers. Conclusion- PAR-2-mediated excitation and sensitization of peripheral primary nociceptors was enhanced by hyper and hypotonic stimuli via TRPV4.

Disclosures: K. Kido: None. **E. Masaki:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

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Topic: D.08. Pain

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NIH Grant NS070711

Title: *In vivo* inflammation causes increased current amplitude in myelinated sensory neurons through mechanosensitive proteins

Authors: *A. M. REYNOLDS, A. D. WEYER, C. L. O'HARA, C. L. STUCKY;
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Abstract: Traditionally, unmyelinated C-fibers and thinly myelinated Aδ fibers are thought to be responsible for achy, dull pain and sharp pain, respectively. However, evidence suggests some heavily myelinated Aβ fibers may encode painful stimuli. Myelinated neuron subtypes have diverse responses to mechanical stimuli, yet the mechanisms underlying the heterogeneity in the responses in the populations are unknown. Furthermore, there is a lack of data on mechanotransduction sensitization after *in vivo* inflammation. To address these questions, we identified subtypes of A fiber-types that possess distinct electrical and chemical properties;

Calcitonin Gene-Related Peptide, CGRP-positive and CGRP-negative large diameter neurons. To distinguish between cutaneous and muscle afferents, a retrograde tracer was used; wheat germ agglutinin conjugated to an Alexa Fluor dye. The retrograde tracer was injected into either the saphenous nerve for cutaneous afferents, or into the gastrocnemius muscle. Cutaneous inflammation was induced with a subcutaneous plantar hindpaw injection of Complete Freud's Adjuvant (CFA), whereas muscle inflammation was produced by either CFA or acid injection into the gastrocnemius muscle. Cutaneous and muscle CGRP-negative and -positive projecting neurons were tested by patch clamping and focal stimulation before and 2 days after inflammation. Before inflammation, both the cutaneous and muscle afferents had similar properties; CGRP-negative neurons had significantly larger rheobases and mechanically-induced currents than CGRP-positive neurons. This differed after inflammation. Cutaneous CGRP-negative neurons showed no mechanical sensitization to inflammation, yet CGRP-positive neurons displayed a 2.5 fold increase in current amplitude. Muscle-projecting large CGRP-negative neurons displayed a 3-fold increase in mechanical current amplitude in response to CFA, whereas CGRP-positive neurons had no sensitization. We used Fluorescence Activated Cell Sorting to differentiate the neurons (CGRP-positive vs CGRP-negative, cutaneous inflamed vs uninflamed, and muscle inflamed vs uninflamed). Transcriptomes were compared between groups using microarrays. None of the upregulated genes were known mechanosensitive proteins, suggesting that there may be unidentified mechanosensitive proteins responsible for the sensitization. Overall we showed for the first time that mechanosensitive proteins can be sensitized after *in vivo* inflammation and that A fiber-type large myelinated neurons can be sensitized.

Disclosures: **A.M. Reynolds:** None. **A.D. Weyer:** None. **C.L. O'Hara:** None. **C.L. Stucky:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

Location: Hall A

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Topic: D.08. Pain

Title: Gender differences in nerve fiber conduction parameters in human and rodent C-nociceptors

Authors: R. SOLÀ, M. SUMALLA, *J. SERRA;
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Abstract: Background: Pain sensitivity is thought to be mediated by a combination of biological, psychological and sociocultural factors. It has consistently been shown that women perceive noxious stimuli as more painful than men. This difference has also been shown in rodents. Although it is known that sex hormones influence pain sensitivity depending on the stage of the menstrual cycle, no electrophysiological study has shown any consistent gender difference in the conduction properties of primary nociceptors. Objective: To test whether peripheral C-nociceptors show any gender difference in their conduction properties, both in humans and in rats. Methods: We measured activity-depending slowing of conduction velocity (ADS) and conduction velocity (CV) in a group of healthy human subjects (n=22, females 12, age 38.9±3.3 years; males 10, age 37.7±3.7 years) and a group of control healthy Sprague-Dawley rats (n=52; females n=25; males n=27) using microneurography from the superficial peroneal nerve or the sciatic nerve, respectively. Results: We recorded a total of 141 C-nociceptors (75 mechano-heat-sensitive [CMH], 66 mechano-insensitive afferents [MIA]) from the humans and 87 C-nociceptors (42 CMH, 45 MIA) from the rats. We detected a striking statistically significant difference in CV of C-nociceptors between males and females in humans (male 0,70 ± 0,18 m/s; female 0,51 ± 0,25 m/s; t-test $P<0.001$) and rats (male 0.81±0.4 m/s; female 0.7±0.02 m/s; $P<0.0046$). Also, dispersion was higher for human females CMH units (SD=0.29) than for males (SD=0.12). Discussion: Our results show a clear gender difference in the conduction velocity of C-nociceptors in human and rat. This difference may be of importance in explaining the higher pain sensitivity of females. The slower CV and greater dispersion of conduction velocities of female C-nociceptors could enhance temporal summation of the peripheral input into the spinal cord. Conclusion: CV of peripheral C-nociceptors is slower in females than in males. This electrophysiological feature could contribute explain differences in pain perception between genders in humans and rodents.

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Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

Location: Hall A

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Program#/Poster#: 703.07/P40

Topic: D.08. Pain

Support: NS023725

Title: Single Cell qPCR analysis of expression changes following nerve injury and regeneration

Authors: *P. C. ADELMAN[1], K. M. BAUMBAUER[2], R. L. FRIEDMAN[1], K. H. LEE[1], H. R. KOERBER[1];
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Abstract: Nerve injury frequently leads to hyperalgesia and allodynia, which depend partially on increased sensitivity in primary sensory afferents to thermal and mechanical stumuli. We have previously characterized functional changes in histologically identified cutaneous afferents following nerve cut and regeneration, and investigated potential molecular mechanisms for the development of hypersensitivity using qPCR on whole dorsal root ganglion (DRG) homogenate. This process was fruitful and indicated several significant changes in expression of genes that were necessary components in the development of hypersensitivity. However, examination of expression levels from whole DRGs potentially misses subpopulation-specific changes that are either eliminated by contrary changes in other subpopulations or diluted to insignificance by the many cells in the DRG unaffected by the manipulation. In this study, we use single cell qPCR to survey the expression profiles of over 160 small diameter DRG afferents backlabeled from the saphenous nerve before and after injury, and identify new targets that may be involved in the development of mechanical and thermal hypersensitivity after injury in specific subpopulations. Our interpretation of this data was informed by transcriptional profiles of more than 40 functionally identified cells collected using our *ex vivo* saphenous preparation. The results obtained in these studies demonstrate expression changes following injury and regeneration, which vary starkly between different subpopulations of cutaneous afferents. For example, peptidergic afferents show significant increases in GFRα1, P2X3, ASIC2, and Piezo2, while non-peptidergic afferents express decreased GFRα2 but increased GFRα3, P2Y1, and P2X3. These results also show that several of the changes detected in whole DRG homogenate PCR were specific to subpopulations of afferents, and that single cell PCR has unmasked novel population-specific changes that are strongly correlated with the development of mechanical and thermal hypersensitivity of specific subsets of cutaneous nociceptors following injury.

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Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Title: Heating of deeper skin layers might detect spontaneously active heat-sensitized nociceptors

Authors: M. I. NEMENOV[1], B. NAMER[2], R. SCHMIDT[3], I.-P. KLEGGETVEIT[4], M. BACKONJA[5], E. JORUM[4], *M. SCHMELZ[6];
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Abstract: Spontaneous pain is cardinal symptom of painful peripheral neuropathy (PPN). However, in preclincal neuropathic pain models mainly mechanical allodynia is used to differentiate painful from non painful neuropathy, though only fraction of the PPN patients develops mechanical allodynia. This disconnect hinders development of novel analgesics. In contrast, spontaneously active (SA) C nociceptive fibers were found in patients with PPN of different origin as well as in rodent neuropathic pain models, but SA of C fiber is not used widely in translational research. Compared to superficial inflammatory pain where SA C fibers are usually sensitized as tested by contact or radiant heat, contact thermode QST demonstrated increased pain thresholds in PPN indicating sensory thermal deficit. However, diode laser C fiber type selective heat stimulation (C-DLss) reaches deeper skin layers as compared to radiant heat (Moeller-Betram et al., 2003). Using such heat stimuli, we found pain thresholds in patients with diabetic and chemotherapy-induced PPN were not increased compared to healthy control. This result could suggest sensitization of deeper located nociceptors to heat. Indeed, the majority of SA fibers in PPN patients were found among heat sensitive, but mechanically insensitive C-fibers (CMi), that are located subepidermally. In patients with diabetic and chemotherapy induced PPN, sensory fibers “die back”, decreasing fiber density. Thus, higher surface temperatures are required to activate the partly degenerated intraepidermal C polymodal nociceptors, but still they might not activate subepidermal SA CMi fibers. Microneurography data from PPN patients show heat sensitization of spontaneously active CMi fibers. Their activation threshold upon radiant heat was only 40°C (median, n=21, 52% heat positive) as compared to a median of 45°C in non-spontaneously active CMi fibers (n=53, 47% heat positive). However, the surface temperature required to active SA C fibers varies substantially due to different depths of sensory endings, steep temperature gradient of radiant heat stimulation and different degree of sensitization. Thus, DLss activating both, superficial and deep cutaneous nociceptors, should uniquely permit direct activation of subepidermal, spontaneously active CMi fibers in animal models of peripheral neuropathy as well as terminals of these nociceptors in the skin of PPN patients. DLss-based C fiber selective QST and C fiber behavioral tests might therefore provide a more reliable biomarker of painful peripheral neuropathy in the clinics and for preclinical tests assessing efficacy of analgesic compounds.

Disclosures: **M.I. Nemenov:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lasmed LLC,

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Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

Location: Hall A

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Program#/Poster#: 703.09/P42

Topic: D.08. Pain

Title: Stimulated CGRP release from GDNF-responsive procine DRG neurons is higher compared to NGF-responsive ones both in somata and neurites, *in vitro*

Authors: S. SAUER[1], A. KLUSCH[2], C. GORZELLANY[4], R. JONAS[3], *P. W. REEH[5], S. SCHNEIDER[4], M. SCHMELZ[3], M. PETERSEN[3];
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Abstract: The neuropeptide CGRP is a marker for heat- and itch-sensing nociceptors. Its release at their peripheral terminals mediates neurogenic inflammation. In rodents, trkA-expressing neurons are mostly CGRP positive, whereas GFRα /cRET expressing neurons are negative or only weakly positive. We investigated CGRP release from outgrown neurites and somata of NGF- and GDNF responsive neurons and growth factor dependent neurite morphology. Dissociated porcine DRG neurons were seeded into the central lumen of a 3-compartment Campenot chamber. Neurites growing into the distal comp. were thus spatially separated from their somata and both could be exposed to different growth factors. Somata and neurites were cultured under three conditions: (I) NGF in central and side comp., (II) NGF in central and GDNF in side comp., and (III) GDNF in the central comp. and no growth factor in the side comp. After 7 d in culture, K+ (60 mM, 5 min) was added either to the central or to the side comp. Thus, in the high K+ containing comp. neuronal structures were stimulated by membrane depolarization whereas in the adjacent comp. presumably via conducted action potentials. CGRP concentration in the supernatant was determined by ELISA. Total length of outgrown neurites was measured from microphotographs. Morphology of neurite endings was visually classified into those with thin tips or pronounced growth cones. CGRP release from neurites in the side comp. could be detected both after central or side comp. K+-stimulation. Likewise, CGRP release from somata could be detected also with both stimulation modes. Under conditions I and

II, CGRP release from the neurites was not significantly different (calc. 1.1 and 0.6 pg/ml/mm neurite length, respectively), but surprisingly, the highest release was found in neurites cultured under condition III (3.2 pg/ml/mm). Neurites from NGF dependent somata developed pronounced growth cones when cultured under GDNF (II) compared to the other conditions. Our results provide evidence that in pig NGF-responsive neurons GDNF availability at the neurite ending regulates neurite morphology, but does not affect CGRP release. Surprisingly, in pig also GDNF-responsive neurons are peptidergic and exhibit even significantly higher CGRP release than in NGF-responsive neurons.

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Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

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Program#/Poster#: 703.10/Q1

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Support: NIH-R01HL122228

Title: Mechanisms of gpcr-gq-induced action potential discharge in vagal c-fiber terminals

Authors: H. SUN[1], S. MEEKER[2], M. KOLLARIK[1], F. RU[2], *B. J. UNDEM[3];
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Abstract: Many inflammatory mediators evoke action potential (AP) discharge in visceral and somatosensory nociceptive C-fibers via activation of GPCRs. At the level of the cell body, TRPV1 and TRPA1 have been implicated as principle depolarizing charge carriers for GPCR-Gq activation. We evaluated the mechanisms underlying GPCR activation-induced AP discharge at the terminals in a vagally innervated mouse (C57B6) lung preparation. An RNAseq analysis specifically of nodose vagal C-fiber neurons in the nodose/jugular ganglia of C57B6 mice revealed that ~70% of the phospholipase C transcripts were of the PLCβ3 isotype. Bradykinin (10 μM) stimulated AP discharge in nodose C-fiber terminals with an average frequency of 14 ± 2 Hz Hz (n=14), and TFLLR (30 μM), a selective protease activating receptor 1 (PAR-1) agonist, stimulated AP discharge at 17 ± 2 Hz. Neither agonist activated the nerve terminals in PLCβ3 -/- animals (n=6). The GPCR-induced AP discharge at the terminals was not different between wild type and trpc3/trpc6 double -/- animals (obtained from Dr. L. Birnbaumer,

NIEHS), nor was different (P>0.1, n= 8) between wild type and trpa1/trpv1 double -/- animals (the response to capsaicin and mustard oil was absent in these animals). The nonselective cation channel blocker ruthenium red (RR) (10 μM) also failed to inhibit the AP discharge in response to the GPCR agonists (P>0.1, n=6), but blocked the capsaicin response. The Clca channel blocker niflumic acid (100 μM) did not inhibit the GPCR activation of the terminals (P>0.1, n=6). By contrast, the K+ channel blocker 4-AP (300-1000 μM, n=4) mimicked the AP discharge evoked by the GPCR agonists. At the cell bodies the PAR1 agonist evoked a strong calcium response in 21% of neurons (232 out of 1084 cells) isolated from mouse nodose ganglia. The TRPA1 antagonist HC-030031 reduced the number of cells responsive to TFLLR by ~50% (to 9.95±3.78%, P<0.001). The TRPV1 blocker AMG 9810 alone had no significant effects on the response, but combined use of HC-030031 and AMG 9810 further reduced the number of cells responding to PAP1 agonist compared to HC-030031 alone (to 5.76±2.39%, P<0.01). We conclude that TRPA1and VI contribute to the GPCR-Gq-induced calcium responses at the soma of vagal sensory neurons. However, the generator potential in vagal C-fiber terminals evoked by GPCR-Gq agonists depends entirely on PLCβ3 but not on TRP-V1, A1, C3, C6, other RR sensitive cation channels, or Cl- channels. It remains possible that inhibition of a resting K+ current is the primary driver of the generator potentials. Our results also suggest that GPCR-Gq-induced activation of cell bodies and nerve terminals in vagal C-fibers may be different.

Disclosures: H. Sun: None. **S. Meeker:** None. **M. Kollarik:** None. **F. Ru:** None. **B.J. Undem:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 703.11/Q2

Topic: D.08. Pain

Support: DoD Grant W81XWH-13-1-0355

Title: Vascular afferents innervating lumbosacral veins have distinct immunohistochemical phenotypes in DiI-traced DRG neurons

Authors: V. HENAO[1], H. D. NGUYEN[1], V. P. DUGAN[1], B. Y. COOPER[2], *R. D. JOHNSON[3];
[1]Physiological Sci., [2]Oral Surgery, [3]Univ. Florida, Gainesville, FL

Abstract: Input from vascular sensory neurons has been implicated in painful conditions including migraine headache and deep tissue pain. Our previous work on DRG neurons used a combined *in vitro* electrophysiology and immunohistochemical phenotyping approach utilizing target-specific tracing from the periphery (skin, muscle, viscera, mucocutaneous, etc.) in the rat lumbosacral region. We have found distinct target-specific phenotypic patterns, and in the present report, extend this investigation to the vasculature. Under aseptic procedures in adult male Sprague-Dawley rats, the proximal segment of the left lateral tail vein was surgically isolated (1.5-2 cm), a glass microsphere luminal plug placed at the cranial end, and the vein sutured closed at the caudal end. The closed-end venous luminal space was filled with a fluorescent DiI-paste delivered through a 26-gauge catheter. After 13 days of tracer transport time, the animals were euthanized and perfused transcardially with 4% paraformaldehyde. The left and right L5-S2 DRGs were dissected free, post-fixed overnight and cryoprotected. The Di-I injection site was examined postmortem to verify that no dye leaked into non-vascular structures. Serial cryosections at 14 μm were thaw-mounted on alternating slides. Nucleated DiI-positive DRG neurons were directly visualized via multi-label fluorescence microscopy, digitally imaged with Zeiss optics, and measured with morphometric software. Using our previously published techniques (e.g. Petruska et al, 2000), slides were immunohistochemically processed for various cellular markers including CGRP, SubP, Neurofilament-M (NF-M), IB4, TRPV1, TRPV2. Di-I positive cells neurons (n=929) traced from the proximal tail vein were found almost exclusively in the ipsilateral L6 and S1 DRGs (some in S2) and exhibited small to medium cell diameters (96% between 15-45 μm). Only 19% were NF-M positive but had a significantly greater cell diameter (41.5 ± 1.1 μm) compared to NF-M negative cells (31.2 ± 0.5 μm; $p<.01$). The latter were mostly IB4 negative. While only 21% were typical peptidergic cells containing both CGRP and SubP, those containing only one peptide were CGRP+ (92%). Combined with our finding that one third of the total Di-I cells contained CGRP, our data support the known importance of CGRP in vascular control. Consistent with our *in vitro* patch clamp data from capsaicin-sensitive and -insensitive Di-I traced vascular DRG neurons (type 8, type 19 and type 20; Cooper et al., 2014), a portion of the vascular afferent population were positive for TRPV1 and TRPV2 with most CGRP+ cells co-labeled with the capsaicin-insensitive TRPV2 receptor (63%).

Disclosures: V. Henao: None. **H.D. Nguyen:** None. **V.P. Dugan:** None. **B.Y. Cooper:** None. **R.D. Johnson:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

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Topic: D.08. Pain

Support: NIH R01 NS023725

NIH R01 AR063772

NIH R21 AR064445

Title: Characterization of optogenetic activation of non-peptidergic C-fibers

Authors: *K. H. LEE[1,2], J. HACHISUKA[1,2], P. C. ADELMAN[1,2], S. E. ROSS[1,2,3], B. M. DAVIS[1,2,4], H. R. KOERBER[1,2];
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Abstract: A critical function of the central nervous system is rapidly detecting harm or injury, signaling peripheral injury by sending pain or nociceptive signaling to the spinal cord and then to the brain to prevent further damage. Using an optogenetic approach that inserts light-activated channels (ChR2) in specific cell types, the current study characterized the afferent inputs that are activated by light in mouse genetic line expressing MrgD-Cre-Ai32, which selectively activate a non-peptidergic population of thermally and mechanically sensitive C-fibers. First, we characterized the behavioral responses to light stimulation, finding a distinct withdrawal of the hindpaw after light presentation to both the dorsal and ventral skin, verifying that light presentation can produce a response behaviorally similar to mechanical and thermal stimulation. Likewise, electrophysiological recordings show that blue light activation can evoke responses in MrgD fibers that mimic those observed following mechanical or thermal stimulation. Next, we examined the potential of these primary afferent inputs to activate lamina I projection neurons back-labeled from the parabrachial nucleus. We used the skin-nerve-DRG-spinal cord *ex vivo* preparation to record from tract cells while optically activating MrgD afferent fibers within the tract cell's receptive field. Preliminary data show that MrgD-Cre-Ai32 expressing light driven afferent inputs both directly and indirectly reach some lamina I tract cells. Finally, to examine the role of this population of non-peptidergic C-fibers in inflammatory pain, we measured response to blue light before and after CFA injection into the hindpaw (intradermal). Interestingly, we found no change in light intensity threshold after CFA injection in MrgD-Cre-Ai32 mice, even though the same mice demonstrated thermal hyperalgesia. Although unlikely, one possible interpretation of these results is that these MrgD fibers, which make up a large proportion of cutaneous nociceptors, are not involved in mechanical or thermal hyperalgesia following inflammation. Another interpretation would suggest that increased sensitivity in sensory transduction plays a major role in the hyperalgesia observed after inflammation rather than a general increase in afferent fiber excitability.

Disclosures: **K.H. Lee:** None. **J. Hachisuka:** None. **P.C. Adelman:** None. **S.E. Ross:** None. **B.M. Davis:** None. **H.R. Koerber:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 703.13/Q4

Topic: D.08. Pain

Title: A newly identified selective mechano-sensitive K2P opener reduces rat dorsal root ganglion (DRG) neuronal excitability

Authors: *A. J. LOUCIF[1], P.-P. SAINTOT[2], B. ANTONIO[3], S. ZELLMER[3], L. CAO[2], N. CASTLE[3], E. STEVENS[2];
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Abstract: TREK-1, TREK-2 and TRAAK channels belong to the mechano-sensitive family of two-pore domain (K2P) potassium channels which are sensitive to a variety of stimuli including temperature, mechanical activation. TREK-1, TREK-2 and TRAAK channels are highly expressed in rat dorsal root ganglia neurones and play a role in setting the threshold and range of nociceptor excitation in response to pain stimuli. Knock-out studies show that TREK-1 and TRAAK channels can be considered as molecular silencers of mechanical pain and both noxious heat and cold pain (Noel et al, 2009), while TREK-2 can be considered as a regulator of mechanical and moderate heat and cold pain (Pereira et al, 2014). In the present study we have characterised the action of a newly identified mechano-sensitive K2P opener in recombinant cell lines and cultured rat DRG neurones. Using manual patch clamp technique on HEK cells stably expressing rat TREK-1, the opener induced an outwardly rectifying current with a maximum enhancement of 5902% and an EC_{50} of 0.9μM (n=6). Using manual patch clamp in recombinant cell lines, little or no effects were observed against other potassium channels at 10 uM: TRESK (-2.0±3.5%, n=4), KCNQ1 (-24.6±8.9%, n=4), BK (-3.3x±6.7%, n=4) and hERG (-7.3±5.1%, n=5). The opener had no effects against other potassium channels TASK-2, KCNQ-1, KCNQ-2, KCNQ-3 and sodium channels Nav1.2, Nav1.7 and Nav1.8 using Rb^+ flux assays. Using current clamp recordings on cultured rat DRG neurones application of compound at 1μM (~EC50) resulted in a significant reduction in firing frequency as a result of a reduction in input resistance and a small hyperpolarisation of resting membrane potential (~2mV). This provides the first pharmacological evidence for the presence of mechano-sensitive K2P channels in sensory neurones and suggests that development of selective K2P channel openers could potentially lead

to novel analgesics. Noël et al., 2009 EMBO J. 28(9):1308-18. Pereira et al., 2014 155(12):2534-44.

Disclosures: **A.J. Loucif:** None. **P. Saintot:** None. **B. Antonio:** None. **S. Zellmer:** None. **L. Cao:** None. **N. Castle:** None. **E. Stevens:** None.

Poster

703. Dorsal Root Ganglion Neuron Modulation and Function

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 703.14/Q5

Topic: D.08. Pain

Title: Elevated levels of glutaminase and aspartate aminotransferase in rat drg neurons during adjuvant-induced arthritis

Authors: *B. R. BOLT, Z. ZHANG, K. E. MILLER;
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Abstract: The sensory neurons of the dorsal root ganglia (DRG) are glutamatergic, utilizing glutaminase (GLS) and aspartate aminotransferase (AST) for glutamate synthesis (Miller et al., Pharmacol Ther 130:283, 2011). We previously demonstrated that glutamate metabolism (↑ GLS, AST, glutamate levels) is altered in the rat DRG, sciatic nerve and skin during adjuvant-induced arthritis (AIA). Additionally, we have determined that AST is elevated in nociceptive, calcitonin gene-related peptide- (CGRP) containing DRG neurons during AIA. The aim of the present study is to evaluate alterations of GLS in AST-containing DRG neurons during AIA. Complete Freund's adjuvant was injected into the rat right hindpaw to induce AIA and DRG's were analyzed at 2, 4 and 8 days. Rats were anesthetized and transcardially perfused with fixative. AST and GLS colocalization was evaluated in L4 DRG's with immunohistochemistry, followed by quantitative image analysis with Image J. At 2 and 8 days AIA, GLS-immunoreactivity (ir) in AST-positive DRG neurons was elevated when compared to naïve controls, demonstrating a biphasic pattern of expression. In a similar biphasic pattern, AST-ir in GLS-positive neurons was elevated at days 2 and 8 of AIA, but was a control levels at day 4 AIA. Analysis demonstrated strong positive correlation in GLS-AST elevation in DRG neurons at 2 and 8 days of AIA. Elevated AST and GLS levels in DRG neuronal bodies leads to increased glutamate production in peripheral and central terminals. The elevation observed for both AST and GLS, therefore, may be due to similar mechanisms regulating transcription, translation, and trafficking in DRG neurons. Novel therapies that decrease AST and GLS levels

may hold promising treatment for alleviating inflammatory pain caused by increased glutamate synthesis in nociceptive primary afferents following injury.

Disclosures: **B.R. Bolt:** None. **Z. Zhang:** None. **K.E. Miller:** None.

Poster

704. Peripheral Mechanisms: Pain and Touch

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 704.01/Q6

Topic: D.08. Pain

Title: Molecular profiling of sensory neurons innervating different peripheral tissues following *in vivo* retrograde labeling and laser capture microdissection

Authors: R. S. BREESE, Y. BAI, W. FURY, Y. WEI, M. NI, C. ADLER, C. LIN, A. J. MURPHY, L. E. MACDONALD, *N. ALESSANDRI-HABER;
Regeneron Pharmaceuticals, Tarrytown, NY

Abstract: Chronic painful conditions affect millions of people worldwide. Unlike the transitory unpleasant sensation of acute pain, inflammatory and neuropathic pain are often chronic, persistent states. Patients suffering from chronic pain often experience hypersensitivity to mechanical, thermal and/or chemical stimulation in the form of hyperalgesia and/or allodynia. This hypersensitivity is mediated, at least in part, by sensitization of signaling processes in different subpopulations of primary sensory neurons known as nociceptors. The cell bodies of these neurons are clustered in nodules on dorsal roots of the spine known as dorsal root ganglia (DRG). The axons of these neurons split into two branches; one branch innervates the peripheral tissues (skin, muscles, joints and organs) and the other connects to the spinal cord to carry signals to the appropriate integration center in the brain. Nociceptors fall into different subpopulations based on their conduction properties, molecular profile and whether they respond to a single type of physical stimulus or integrate and generate a response to different types of physical stimuli. Although the development of chronic pain has been associated with increased excitability of nociceptors, their molecular complexity has made it difficult to fully elucidate the mechanisms underlying persistent pain. We hypothesized that molecular profiling of subpopulations of nociceptors innervating distinct peripheral tissues in different animal models of pain would improve our understanding of the mechanisms underlying the establishment of chronic pain and potentially unravel new targets for pain therapeutics. Thus, we developed a technique to isolate neurons innervating peripheral tissues using laser microdissection after *in vivo* retrograde labeling. We first determined which DRGs innervated the gastrocnemius muscle,

the skin of the footpad, the knee joint or the femoral bone using fluorescently labeled Cholera toxin subunit B. Retrograde tracers clearly showed that primary afferent neurons originated from multiple DRG levels along the spine with a pattern specific to each peripheral tissue. We then investigated the molecular profiles of DRG neurons innervating peripheral tissues in two animal models of pain; a model of inflammatory muscle pain (repeated acid injection into the gastrocnemius muscle) and a model of knee joint inflammation (Complete Freud's Adjuvant injection into the knee joint). We show here that laser capture microdissection of retrogradely labeled sensory neurons in combination with RNA-seq can successfully be used for molecular profiling of specific subpopulations of nociceptors.

Disclosures: **R.S. Breese:** A. Employment/Salary (full or part-time):; Full time, Regeneron Pharmaceuticals, Inc. **Y. Bai:** A. Employment/Salary (full or part-time):; Full Time, Regeneron Pharmaceuticals, Inc. **W. Fury:** A. Employment/Salary (full or part-time):; Full time, Regeneron Pharmaceuticals, Inc. **Y. Wei:** A. Employment/Salary (full or part-time):; Full time, Regeneron Pharmaceuticals, Inc. **M. Ni:** A. Employment/Salary (full or part-time):; Full time, Regeneron Pharmaceuticals. **C. Adler:** A. Employment/Salary (full or part-time):; full time, Regeneron Pharmaceuticals, Inc. **C. Lin:** A. Employment/Salary (full or part-time):; full time, Regeneron Pharmaceuticals, Inc. **A.J. Murphy:** A. Employment/Salary (full or part-time):; Full time, Regeneron Pharmaceuticals, Inc. **L.E. Macdonald:** A. Employment/Salary (full or part-time):; full time, Regeneron Pharmaceuticals Inc. **N. Alessandri-Haber:** A. Employment/Salary (full or part-time):; full time, Regeneron Pharmaceuticals, Inc.

Poster

704. Peripheral Mechanisms: Pain and Touch

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 704.02/Q7

Topic: D.08. Pain

Support: NIH R01GM102346

Title: Flotillin 1 is enriched in nociceptive sensory neurons and its interactome is altered in response to inflammation

Authors: B. K. DRAGOO, R. GEGUCHADZE, *D. C. MOLLIVER;
Biomed. Sci., Univ. New England, Biddeford, ME

Abstract: Flotillins 1 and 2 are ubiquitously-expressed membrane-associated proteins that segregate in specialized membrane "lipid raft" cholesterol-rich compartments. Flotillins appear

to act as scaffolding proteins that organize signal transduction complexes associated with raft microdomains and may mediate interactions between the plasma membrane and the cytoskeleton. Roles of flotillins as well as their binding partners are likely to vary based on cell type. Here, we examined the distribution of flotillin 1 and 2 in sensory neurons of the mouse dorsal root ganglia (DRG) and their central projections in the spinal cord. As expected, immunohistochemical staining for both proteins was ubiquitous in neuronal cell bodies. However, in contrast to flotillin 2, which was homogenously distributed, flotillin 1 showed a range of intensities in different neurons and was markedly more intense in small diameter neurons positive for the plant lectin IB4 and the heat-gated ion channel TRPV1, markers commonly associated with nociceptors. Furthermore, although flotillin 1 staining was widespread across the lumbar spinal cord, labeling was much more intense in the superficial dorsal horn (the principal target region for unmyelinated nociceptors), and colocalized with staining for both IB4 and TRPV1. A second polyclonal antibody generated against a different region of the protein yielded essentially identical results and staining was eliminated by incubation of the primary antibody with the antigenic peptide. These results are consistent with a high level of flotillin 1 expression in nociceptors compared to other sensory neurons. Western blot analysis of lumbar L2-5 DRG of mice injected with either vehicle or complete Freund's adjuvant (CFA) to produce inflammatory hyperalgesia did not reveal substantial changes in flotillin 1 levels in response to inflammation. To identify proteins interacting with flotillin 1 in DRG under normal and inflamed conditions, we performed flotillin 1 coimmunoprecipitation from lumbar DRG of mice treated with vehicle or CFA. Eluted samples were compared by 2 dimensional difference gel electrophoresis (2D DIGE). In preliminary analysis of naive samples, 14 unique protein spots were identified, of which 2 were highly enriched in the eluted fraction compared to the lysate flow-through fraction. Twelve spots were identified in inflamed samples, 2 of which were distinct from those identified in naïve samples. All spots are undergoing protein sequencing by MALDI-Tof mass spectrometry. These results suggest that signaling complexes organized by flotillin 1 at the sensory neuron plasma membrane are altered in response to inflammatory injury.

Disclosures: B.K. Dragoo: None. **R. Geguchadze:** None. **D.C. Molliver:** None.

Poster

704. Peripheral Mechanisms: Pain and Touch

Location: Hall A

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Topic: D.08. Pain

Support: NS040538

NS070711

Title: Cage bedding material affects mechanical behavioral thresholds, heat thresholds and texture preference in mice

Authors: *F. MOEHRING, C. L. O'HARA, C. L. STUCKY;
Cell Biology, Neurobio. and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: It has long been known that the bedding material animals are housed on can affect breeding behavior and cage environment. Yet little is known about its effects on evoked behavior responses or non-reflexive behaviors. In recent years, we noticed that the median von Frey thresholds obtained by our laboratory from non-injured wild type mice are typically lower (2-3 fold) than those obtained by other groups. Therefore we housed C57bl6 mice for two weeks on various bedding types (Aspen Sani Chips® (standard bedding for our institute), ALPHA-Dri®, Cellu-Dri™, Pure-o'Cel™ or TEK-Fresh bedding) and tested the animals with an array of behavior assays. Mice housed on Aspen bedding exhibited the lowest mechanical thresholds, while those housed on TEK-Fresh exhibited 3-fold higher thresholds. TEK-Fresh housed animals also exhibited greater responsiveness in a noxious needle assay, whereas bedding type had no effect on responses to punctate or dynamic light touch stimuli. Heat sensitivity was also affected by bedding; animals housed on Aspen Sani Chips® exhibited the shortest latencies to withdrawal, whereas those housed on TEK-Fresh had the longest latencies to response; similar results were observed in a moderate cold temperature preference assay. A modified tactile conditioned place preference chamber assay revealed that animals preferred TEK-Fresh to Aspen bedding. Bedding type had no effect in a non-reflexive wheel running assay. Two days after peripheral inflammation induced by injection of Complete Freund's Adjuvant (CFA) in the hindpaw, mechanical thresholds were reduced in all groups regardless of bedding type, but TEK-Fresh and Pure-o'Cel™ housed groups exhibited a greater dynamic range between controls and inflamed cohorts as compared to Aspen housed mice. For the Hargreaves test, all animals receiving CFA had shorter heat withdrawal latencies, however the bedding type did not significantly affect the dynamic range. These findings indicate that the bedding type that animals are routinely housed on can markedly affect the dynamic range of mechanical and heat behavior assays under both normal, non-injured and tissue injury conditions. Therefore, careful consideration should be used when selecting bedding material, because a softer bedding material may allow for the utilization of fewer animals through less intra-animal variability, and therefore decrease the time and money required for somatosensory studies.

Disclosures: F. Moehring: None. **C.L. O'Hara:** None. **C.L. Stucky:** None.

Poster

704. Peripheral Mechanisms: Pain and Touch

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NIHGrant R03HD077483-01

Title: Growth hormone regulates the age-dependent sensitization of cutaneous nociceptors during developmental inflammation through an insulin-like growth factor receptor type 1 dependent mechanism

Authors: *X. LIU[1,2], L. QUEME[1], P. LU[1], K. GREEN[1], F. LEE[1], A. SHANK[1], R. HUDGINS[1], M. JANKOWSKI[1,3];
[1]Cincinnati Children's Hosp., Cincinnati, OH; [2]Shaanxi Univ. of Chinese Med., Xi'an, China; [3]Univ. of Cincinnati, Cincinnati, Cincinnati, OH

Abstract: We have previously found that cutaneous nociceptors are age-dependently sensitized to heat and mechanical stimuli in neonatal mice during inflammation of the hairy skin. The patterns of sensory neuron gene expression likely underlying alterations in afferent function were also found to be age-dependent. We therefore sought to determine if there was a common upstream modulator of peripheral sensitization that age-independently regulated neonatal pain. Previous reports have shown that up to 55% of children with growth hormone deficiency (GHD) have resting pain in their limbs and treatment with GH relieves their pain. In order to determine if GH signaling played a role in modulating sensory neuron function and pain during developmental inflammation in mice, we examined the response properties of cutaneous afferents using an *ex vivo* hairy skin-saphenous nerve-dorsal root ganglion (DRG)-spinal cord preparation and also tested behavioral hypersensitivity. Analyses were performed one day after injection of 3% carrageenan into the hairy hindpaw skin at postnatal days 7 and 14 in mice with or without GH treatment and compared to naïves. We found that cutaneous inflammation produced a GH deficient state in the affected skin and treatment of inflamed mice with exogenous GH reversed mechanical and thermal hypersensitivity. In addition, all of the age-dependent alterations in cutaneous afferents were blocked by this GH treatment in mice with cutaneous inflammation. Since GH is known to mediate its actions by regulating the insulin-like growth factor 1(IGF1)/IGF receptor type 1 (IGFr1) pathway, we wanted to determine if a similar mechanism of GH action was found in primary afferents. We found that the GHD produced in

the skin during developmental inflammation induced a compensatory upregulation of IGFr1 in the DRGs. Afferent selective, siRNA-mediated knockdown of IGFr1 during neonatal inflammation also prevented the observed age-dependent alterations in cutaneous afferents in addition to mechanical and thermal hypersensitivity. These results suggest that GH blocks the inflammation induced changes in thermal and mechanical sensitivity in cutaneous afferents at different postnatal ages leading to reduced pain behaviors through an IGFr1 dependent mechanism in the DRGs.

Disclosures: X. Liu: None. **L. Queme:** None. **P. Lu:** None. **K. Green:** None. **F. Lee:** None. **A. Shank:** None. **R. Hudgins:** None. **M. Jankowski:** None.

Poster

704. Peripheral Mechanisms: Pain and Touch

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Rita Allen Foundation/American Pain Society

International Association for the Study of Pain Early Career Grant

Title: Muscle IL-1β mediates ischemia and reperfusion injury-induced sensitization of group III and IV muscle afferents

Authors: *J. L. ROSS[1], L. F. QUEME[1], P. LU[1], E. R. COHEN[1], R. C. HUDGINS[1], M. P. JANKOWSKI[2];
[1]Anesthesia, [2]Anesthesia, Pediatrics, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: We have previously shown that ischemia and reperfusion injury (I/R) in the forepaw muscles of adult Swiss Webster mice altered sensory behaviors and muscle function *in vivo*. I/R also decreased mechanical thresholds and increased firing to chemical stimuli in group III and IV muscle afferents as assessed by an *ex vivo* muscle-nerve-dorsal root ganglion (DRG)-spinal cord recording preparation. Additionally, I/R induced a significant increase in the number of muscle afferents that responded to both non-noxious (low lactic acid and ATP concentrations; pH 7.0) and noxious (high lactic acid and ATP concentrations, pH 6.6) metabolite solutions. These functional changes were thought to be mediated through an acid sensing ion channel 3 (ASIC3)-dependent mechanism in the affected sensory neurons. We have recently found that the

inflammatory cytokine interleukin 1β(IL1β) was upregulated in the injured muscles and its receptor IL1r1 displayed enhanced expression in the DRGs after I/R. IL1β treatment of dissociated muscle afferents also significantly increased expression of ASIC3 in single cells. Therefore, to further determine the mechanistic role of IL1β in the generation of muscle pain after I/R, we coupled our injury model with an afferent-specific siRNA knockdown of IL1r1, and performed molecular, electrophysiological and behavioral analyses. Not only did nerve specific IL1r1 knockdown inhibit I/R-induced upregulation of IL1r1, but it also inhibited the upregulation of ASIC3 expression in the affected DRGs *in vivo*. Furthermore, *ex vivo* recording revealed that knockdown of IL1r1 blocked the I/R-induced changes in mechanical thresholds and in the altered prevalence of chemosensitive muscle afferents. siRNA-mediated inhibition of IL1r1 also blocked I/R-induced alterations in spontaneous (guarding) and evoked (von Frey mechanical stimulation) pain behaviors, as well as attenuating I/R-induced deficits in grip strength and voluntary activity. This suggests that enhanced IL1β in the muscle during I/R upregulates ASIC3 expression in muscle afferents via IL1r1, altering neuronal responsiveness and phenotypic identity, and leading to the development of muscle pain. These data provide a potential peripherally restricted therapeutic target for the treatment of ischemic myalgias.

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Poster

704. Peripheral Mechanisms: Pain and Touch

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MRC grant G1002183

MRC grant G0700966/82507

Title: Ionic mechanisms of peripheral analgesic effect of Substance P

Authors: *N. GAMPER[1,3], D. HUANG[3], L. OOI[1], J. LINLEY[1], C. PEERS[2], H. ZHANG[3]; [2]Fac. of Med. and Hlth., [1]Univ. Leeds, Leeds, United Kingdom; [3]Dept. of Pharmacol., Hebei Med. Univ., Shijiazhuang, China

Abstract: Neuropeptide substance P (SP) is both produced and released by a subset of peripheral sensory neurons that respond to tissue damage (nociceptive neurones); these neurons also express SP receptors (neurokinin receptors 1-3, NK1-3). SP exerts excitatory effects in the CNS but peripheral SP actions are still under investigation. We show that SP inhibits excitability of small-diameter dorsal root ganglion (DRG) neurons *in vitro* and produces a peripheral analgesic effect *in vivo*. Furthermore, we demonstrate that this effect can be attributed to the acute modulation of two ion channels that control sensory neuron excitability: potentiation of anti-excitatory M-type K+ channels and inhibition of pro-excitatory T-type voltage-gated Ca2+. Both effects were sensitive to Gi/o inhibitor pertussis toxin and were mediated by NK1 receptor-induced stimulation of intracellular release of reactive oxygen species (ROS) as these were prevented or reversed by the reducing agent dithiothreitol and mimicked by exogenous or endogenous ROS delivery. Fluorescent imaging utilizing superoxide-sensitive fluorescent protein mt-cpYFP revealed that in DRG neurons SP induced mitochondrial release of superoxide radical, which explains the action of SP on both channel types as both are redox-sensitive (although in opposite directions). Focusing on the T-type channels, we further demonstrate that the SP-induced and redox-mediated T-type channel inhibition in DRG neurons operated through the modulation of Cav3.2 channel sensitivity to ambient zinc as it was prevented or reversed by zinc chelation and mimicked by exogenous zinc. Moreover, elimination of the zinc binding site in Cav3.2 rendered the channel insensitive to SP-mediated inhibition. Using behavioural tests we demonstrate that peripheral injections of SP into rat hind paw produced no nocifensive behaviour but pre-injection of SP significantly attenuated nocifensive behaviour produced by injection of inflammatory mediator bradykinin. This effect of SP was partially mimicked by the peripheral injections of the M channel opener retigabine and by the T-type channel inhibitor Z944. Thus, our study establishes a convergent mechanism by which SP exerts its peripheral analgesic effect by simultaneous inhibition of pro-algesic T-type Ca2+ current and enhancement of anti-algesic M-type K+ current. These findings will lead to better understanding of mechanisms of endogenous analgesia.

Disclosures: **N. Gamper:** None. **D. Huang:** None. **L. Ooi:** None. **J. Linley:** None. **C. Peers:** None. **H. Zhang:** None.

Poster

704. Peripheral Mechanisms: Pain and Touch

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Topic: D.08. Pain

Support: NIH Grant USPHS NS078173

Title: Translation of the atypical protein kinase C, PKMζ, mediates the enhancement of excitability and the development of mechanical hyperalgesia produced by Nerve Growth Factor

Authors: *A. KHODOROVA[1], J. KAYS[2], Y.-H. ZHANG[2], S. YOUNG[3], R. WEK[3], G. R. STRICHARTZ[1], G. D. NICOL[2];
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Abstract: PKM ζ is a key effector in the maintenance of long-term memory. Our recent studies indicate that an atypical PKC, PKM ζ, contributes to nociceptive sensitization by NGF (Zhang et al., 2012; Khodorova et al., 2013; SfN 2014). Here we evaluate whether these sensitizing effects result from the translation of PKM ζ. We found that pre-treatment of rat sensory DRG neurons with inhibitors of protein translation cyclohexamide (CHX) or rapamycin (RAPA) prevented the increase in excitability produced by exposure to 100 ng/ml NGF, whereas actinomycin-D, a transcription inhibitor, had no effect. Moreover, pre-treatment with 4EGI-1, a small molecule inhibitor of cap-dependent translation (specifically blocks the interaction between eIF4E and eIF4G), also blocked the NGF-induced increase in excitability. In polysomal profiling experiments on isolated sensory neurons, NGF significantly augmented the amount of mRNA for PKM ζ in the peak polysomal fraction, and increased the ratio of the mRNA content of the combined polysomes to the combined monosomes. No increase was detected for other atypical PKCs (PKC ζ or PKCλ/ι). Importantly, RAPA reduced the levels of PKM ζ mRNA found in the peak polysome fraction to values measured in untreated control neurons. Western blotting showed that exposure of the neurons to 100 ng/ml NGF (30 min) significantly increased the density of the ~49 kDa band, previously identified as PKM ζ; a 1 h pre-treatment with CHX reduced the density of this band to untreated control levels. In rats, either CHX or RAPA, pre-injected before NGF into the plantar hind paw, delayed the development of mechano-hyperalgesia at that locus. A significant hypersensitivity from NGF (500 ng) was not detected until 3.5 h or later after CHX, or RAPA, with the mechanical hypersensitivity returning to the normal NGF-elevated value by 22 h. RAPA pre-treatment also decreased the NGF-induced thermal hyper-responsiveness, but only at the 1 h time (compared to responses of vehicle pre-treated controls). Intraplantar injection of the pseudosubstrate inhibitor of the atypical PKCs (mPSI) or RAPA 3 days after NGF treatment (1-4 μg), reversed the persistent mechano-hypersensitivity measured 1 day later. By day 5 the responsiveness had recovered to its enhanced, pre-mPSI level. These results demonstrate that the ability of NGF to sensitize sensory neurons and enhance painful sensitivity in rat glabrous skin is mediated by the protein translation of PKM ζ and that the persistent hypersensitivity may depend, in part, on the continued synthesis of PKM ζ.

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Poster

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Topic: D.08. Pain

Support: National Natural Science Foundation of China (Nos. 81200604, 30400131)

Title: Peripheral glutamate receptors contribute to Fos expression in the spinal dorsal horn through interaction between peripheral nerve terminals

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Abstract: Glutamate receptors localize on the peripheral terminals of primary afferent neurons. Exogenous glutamate can excite peripheral sensory nerve terminals and electrical stimulation of primary afferents evokes the release of glutamate peripherally. Our previous studies have shown that spontaneous nerve discharges increase in a cutaneous branch from a spinal dorsal ramus in the thorax following antidromic electrical stimulation (ADES) of a cutaneous branch at the adjacent spinal segment. The enhanced activity can be blocked by local application of NMDA and non-NMDA receptor antagonists into the receptive field of the recorded nerve. It is well known that the dorsal cutaneous branches of the thoracic nerves run parallel to each other and there is no synaptic contact at the peripheral. The aim of the present study was to investigate whether endogenous glutamate with its receptors has a role in the interaction between the peripheral nerve terminals thus inducing Fos protein expression in the dorsal horn of spinal cord at adjacent spinal segments. Male rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally). A left paramedian incision at 1 cm lateral to the medline on dorsal skin was made longitudinally to expose T10 right dorsal cutaneous branch. ADES (amplitude 0.6 mA, duration 0.5 ms, frequency 20 Hz, lasted for 30 min) was applied on T10 dorsal cutaneous branch. Rats were perfused with 4% paraformaldehyde 1.5 h after ADES. The T8-T12 spinal cord segments were removed, 30-mm sections cut, and every fourth section labeled for c-Fos using standard immunocytochemical protocols. The Fos exprssion in ADES rats was significantly higher than that in the sham ADES rats in the ipsilateral and contralateral dorsal horn of T8-T12 spinal cord. In separated groups, subcutaneous injection of NMDA receptor

antagonist MK-801 or non-NMDA receptor antagonist DNQX (0.1 mmol/L, 30 μl/30min for each drug) into the receptive field of T11 dorsal cutaneous branch during ADES blocked the Fos expression in T11-T12, but not in T8-T10 spinal cord dorsal horn. As the increase of Fos expression is the indication of central sensitization in the spinal cord, these results suggest that endogenic glutamate and its receptors contribute to peripheral sensitization inducing central sensitization in the spinal cord at adjacent spinal segments.

Disclosures: **A. Li:** None. **D. Cao:** None. **Y. Guo:** None. **Y. Zhao:** None.

Poster

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NIH Grant DE017813

Title: P38 MAPK activation is required in glial P2X7-dependent and neuronal P2Y1-mediated inhibition of P2X3 expression in dorsal root ganglion neurons

Authors: *Y. CHEN, G. LI, L.-Y. HUANG;
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Abstract: We have previously shown that activation of purinergic P2X7 receptors (P2X7Rs) in satellite glial cells (SCGs) of dorsal root ganglia (DRGs) stimulate ATP release. The ATP activates P2Y1Rs located in the enwrapped neuronal somata, resulting in down-regulation of P2X3Rs. This P2X7R-P2Y1-P2X3R inhibitory control significantly reduces P2X3R-mediated nociceptive responses. The underlying mechanism by which the activation of P2Y1Rs inhibits the expression of P2X3Rs has not been explored. Examining the effect of activation of p38 mitogen-activated protein kinase on the expression of P2X3R in DRGs, we found that the p38 activator, anisomycin (Anis), reduced the expression of P2X3Rs. The effect of Anis was not altered when the activity of SGCs was blocked by the glial Krebs cycle inhibitor, fluorocitrate. These results suggest that neuronal p38 plays a major role in controlling the P2X3R expression. Antagonizing P2Y1R by MRS2179 (MRS) and blocking P2X7Rs by A740003 inhibited the phosphorylated p38 and increased the P2X3R expressions in DRGs.These observations suggest that activation of P2X7Rs and P2Y1Rs promotes p38 activity to exert inhibitory control on

P2X3R expression. Since activation of p38 by Anis in the presence of either A740003 or MRS could overcome the block of P2X7R-P2Y1R inhibitory control, p38 in DRG neurons is downstream of P2Y1Rs. In addition, inhibition of p38 by SB202190 was found to prevent the P2X7R-P2Y1R block of P2X3R expression and increase P2X3R-mediated nociceptive flinch behaviors. These results led us to conclude that P38 in DRG neurons downstream of P2Y1R is necessary and sufficient for the P2X7R-P2Y1R inhibitory control of P2X3R expression.

Disclosures: Y. Chen: None. **G. Li:** None. **L. Huang:** None.

Poster

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Finnish Academy grant 277442 to RG

Title: Effect of bisphosphonates and bisphosphonates-induced ATP analogues on P2X receptors

Authors: Y. ISHCHENKO, A. SHAKIRZYANOVA, R. GINIATULLINA, P. TURHANEN, J. MÄÄTTÄ, J. MÖNKKÖNEN, *R. GINIATULLIN;
Univ. of Eastern Finland, Kuopio, Finland

Abstract: Accumulating evidence suggests involvement of ATP-gated P2X3 receptors in bone cancer pain. Bisphosphonates are widely used anti-cancer drugs, which also show analgesic effects. Using patch-clamp technique and ATP biochemical assays, we explored molecular mechanisms of the potential direct action of two different bisphosphonates and the bisphosphonate zoledronate-induced metabolite such as triphosphoric acid 1-adenosine-5’-yl ester 3-(3-methylbut-3-enyl) ester (ApppI) on ATP release, on ATP-gated P2X3 and P2X7 receptors expressed in nociceptive system. We found that ApppI inhibited pro-nociceptive P2X3 receptors in low nanomolar concentrations. Unlike ApppI, we established that its precursor isopentenyl pyrophosphate (IPP) known as the inhibitor of TRPV3 and TRPA1 channels (Bang at al., 2011) did not show any activity at the P2X3 receptor consistent with a specific inhibitory potency of the full ApppI molecule. We further demonstrated selectivity of ApppI action on P2X3 receptor by lack of its activity on P2X2 or P2X7 receptors. In line with the specific blocking activity of ApppI, the bisphosphonates zoledronate or clodronatedid not act on P2X3 or

P2X7 receptors directly. Importantly, on human P2X3 receptors the inhibitory action of ApppI was even stronger than on the rat homologue. In addition, no changes in extracellular ATP were found after short (2 h) treatment with zoledronate whereas after 24 h treatment with this bisphosphonate the level of extracellular ATP was significantly enhanced without changes in intracellular ATP content. In contrast, clodronate induced no changes in the level of ATP. We propose that ApppI generated after cancer treatment with nitrate-containing bisphosphonates (like zoledronic acid) can contribute to pain relief via selective ApppI-mediated inhibition of ATP-gated P2X3 receptors natively expressed in nociceptive sensory neurons.

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Poster

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Topic: D.08. Pain

Support: FAPESP Grant #2013/08678-6

Title: A possible role for P2X7 receptors expressed by satellite glial cells from dorsal root ganglia in nociceptive responses

Authors: *A. F. NEVES[1], F. S. OLIVEIRA[2], C. M. C. LOTUFO[3], C. A. PARADA[1]; [1]Dep Structural and Functional Biol., Biol. Inst. / UNICAMP, Campinas, Brazil; [2]Fac. of Med. - UFU, Uberlandia, Brazil; [3]Inst. of Biomed. Sci. / UFU, Uberlandia, Brazil

Abstract: P2X7 purinergic receptors (P2X7R) are selective cation channels activated by ATP that may play an essential role in inflammatory pain. P2X7R are expressed mainly by immune and glial cells. In dorsal root ganglia (DRG), these receptors are expressed by satellite glial cells (SGC). Studies indicate that electrical stimulation of DRG neurons promotes vesicular ATP release from their soma and this could be the major transmitter used for neuron-SGC communication in sensory ganglia. Glial cells are non-electrically excitable, however they could display excitability based on changes in intracellular Ca2+ concentration ([Ca2+]i). Then our aim was to verify if P2X7R activation in SGC are involved in neuronal-SGC communication during nociception. P2X7R activation was tested in primary cultures of adult rat DRG (n=3-5 cultures/group). Changes in [Ca2+]i were assessed by confocal microscopy using Fluo-3 AM (5 μM). P2X7R was activated by BzATP (100 μM) and the TRPV1 agonist capsaicin (1 μM) was

used in order to selectively activate nociceptive neurons. P2X7R was blocked by antagonist A740003 (1 μM), added to cultures 5 min before BzATP or capsaicin. Histological characterization was performed by immunofluorescence in paraformaldehyde-fixed rat DRG using: rabbit anti-P2X7R (1:200, Alomone), goat anti-TRPV1 (nociceptive neuronal receptor; 1:100, R&D System), mouse anti-glutamine synthetase (glial specific enzyme; 1:200, Millipore) and Alexa Fluor conjugated secondary antibodies (1:1000, Invitrogen). Nuclei were stained with DAPI (SIGMA). *In vitro*, BzATP increases [Ca2+]i in SGC but not in neurons. As expected, capsaicin induced a [Ca2+]i surge in nociceptive neurons, but we also observed a calcium transient in SGC about 5 seconds after neuronal response. When cultures were treated with A-740003, the responses of SGC both from BzATP or capsaicin were blocked. The P2X7R antagonist did not affect capsaicin-induced responses in neurons. This result corroborate with the hypothesis that capsaicin responses observed in SGC are indirect through ATP release. Immunofluorescence showed several nociceptive neurons TRPV1-positive and a co-staining of P2X7R and glutamine synthetase surrounding neurons. DAPI staining allowed differentiating neuronal membrane from SGC. This result confirmed that P2X7R are localized only in SGC surrounding most neurons in the DRG. Concluding, the present study suggests that, in the DRG, activated nociceptive neurons release ATP, which activates surrounding SGC. Therefore our data indicate that ATP, through P2X7R, might be involved in the communication between neurons and SGC during nociceptive responses.

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Poster

704. Peripheral Mechanisms: Pain and Touch

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Topic: D.08. Pain

Support: GM102575

Title: *In vivo* regulation of P body dynamics in mouse dorsal root ganglion neurons by AMPK activation and peripheral nerve injury

Authors: ***G. L. MEJIA, JR**[1], K. FIOCK[1], O. K. MELEMEDJIAN[2], G. DUSSOR[1], T. J. PRICE[1];
[1]Univ. of Texas At Dallas, Richardson, TX; [2]Univ. of Maryland, College Park, MD

Abstract: *In vivo* regulation of P body dynamics in mouse dorsal root ganglion neurons by AMPK activation and peripheral nerve injury. Galo L Mejia[1], Kimberly Fiock[1], Ohannes K Melemedjian[2], Gregory Dussor[1], Theodore J Price[1] [1]University of Texas at Dallas, School of Behavioral and Brain Sciences [2]University of Maryland, School of Dentistry, Department of Neural and Pain Sciences P bodies are cytoplasmic RNA granules and hotspots for mRNA turnover. Their specific role in neurons is not known but they have been hypothesized to act as sites of mRNA decay and/or mRNA storage in response to cellular stress and activation of signaling pathways. A function for P bodies in pathology of the nervous system has not been described in detail. We have previously shown that stimuli that increase translation in dorsal root ganglion (DRG) neurons decrease P bodies and activation of signaling pathways that decrease translation (e.g. adenosine monophosphate-activated protein kinase (AMPK) activation) increase P body formation. However, this work has thus far been limited to *in vitro* findings in DRG neurons. To query regulation of P bodies in DRG neurons *in vivo* we treated mice with the AMPK activator metformin for 7 days at a dose of 200 mg/kg. This treatment regimen led to a robust increase in P body formation in DRG neurons. We then asked if peripheral nerve injury with the spared nerve injury (SNI) model in mice altered P body dynamics. We hypothesized that SNI should decrease P body formation because our previous results have shown that SNI increases translation and translation control signaling in DRG neurons. We observed results consistent with this hypothesis 14 days after SNI surgery. Ongoing experiments are examining whether these effects also occur in axons and whether SNI effects on P bodies are reversed by metformin treatment. These findings provide support for a role of P bodies in regulating mRNA turnover and translation control in DRG neurons *in vivo*.

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Poster

704. Peripheral Mechanisms: Pain and Touch

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Support: Conacyt, grant CB-2012/179294 (VG-S)

Conacyt fellows (PB-I and JBP-F)

Title: Pronociceptive role of metabotropic P2Y1 receptor in different models of neuropathic pain

Authors: *V. GRANADOS-SOTO[1], J. B. PINEDA-FARIAS[2], P. BARRAGAN-IGLESIAS[2]; [1]Dept. de Farmacobiologia, Cinvestav, Sede Sur, Mexico, DF, Mexico; [2]Dept. de Farmacobiologia, Cinvestav, Sede Sur, Mexico, DF, Mexico

Abstract: P2Y1 receptor (P2Y1R) is a member of the P2Y family of Gq protein-coupled receptors expressed in peripheral sensory neurons and spinal cord. It has been suggested that P2Y1R has an important role in nociception and inflammatory pain. However, its role in neuropathic pain remains elusive. Here, we investigated the role of the P2Y1R in the modulation of neuropathic pain by using the spinal nerve ligation (SNL), chronic constriction of sciatic nerve (CCI) and spared nerve injury (SNI) models. By Western blot, we found that P2Y1R protein is constitutively expressed in L4-L6 DRGs and lumbar spinal cord. However, the time course of P2Y1R protein expression in L4-L6 ipsilateral DRGs following SNL, CCI or SNI was quite different. SNL and CCI models produced a significant up-regulation of P2Y1R in DRG, but not in spinal cord, at 3 days after nerve injury. On the other hand, SNI produced an early strong up-regulation in DRG, but not in spinal cord, at 1 day post-injury. Intrathecal administration of MRS2500 (19-190 ng/rat) at 3 days (in SNL and CCI models) attenuated tactile allodynia. Moreover, intrathecal administration of MRS2500 (19-190 ng/rat) produced a marked antiallodynic effect in the SNI model 1 day post-injury. Importantly, the antiallodynic effect of MRS2500 (19-190 ng/rat) was lower when it was intrathecally administered at day 14, compared to day 1 and 3 post-injury. The antiallodynic effect of MRS2500 (190 ng/rat) at 3- or 1-day post-injury was associated with a reduction of P2Y1R protein expression in DRG in all models. Results suggest that peripheral and spinal activation of P2Y1R contributes to initiation and, at a lesser extent, to maintenance of neuropathic pain. Furthermore, the pronociceptive effect of P2Y1R depends on the time course of protein expression and the type of nerve injury produced in peripheral sensory nerves.

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Poster

704. Peripheral Mechanisms: Pain and Touch

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Topic: D.08. Pain

Support: SOD NISHIM69749

Title: Differential accumulation of Nav1.8 mRNA with de novo long 3'UTR in sensory axons after peripheral nerve injury

Authors: ***T. HIRAI**[1,2], Y. MULPURI[3], I. SPIGELMAN[2], I. NISHIMURA[2,4];
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Abstract: Axonal transport of mRNA associated with mechanism of neuropathic pain remains unclear in peripheral sensory neuron. We found that Nav1.8 mRNA with novel long 3' untranslated region (UTR), but not conventional short 3'UTR, is exclusively transported and accumulates in peripheral axon using RNA-seq and 3'RACE-PCR. Additionally, Nav1.8 mRNA with the long 3'UTR increases after nerve injury, indicating that this transcriptional variant might be a target of neuropathic pain.

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Poster

704. Peripheral Mechanisms: Pain and Touch

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Support: NS065926

Title: eIF4E phosphorylation links BDNF translation to pathological pain plasticity

Authors: ***J. K. MOY**[1], A. KHOUTORSKY[2], M. N. K. ASIEDU[1], C. G. GKOGKAS[3], G. DUSSOR[1], T. J. PRICE[1];
[1]Univ. of Texas At Dallas, Richardson, TX; [2]Biochem., McGill Univ., Montreal, QC, Canada; [3]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Translational control of gene expression is a key process for the regulation of plasticity in the nervous system. Multiple lines of evidence indicate that translation control plays a critical role in pathological pain plasticity but precise gene targets have thus far not been elucidated. We have previously shown that brain-derived neurotrophic factor (BDNF) is required

for the initiation and maintenance of hyperalgesic priming, a model of pathological pain plasticity. Mice harboring a mutation in eIF4E at Ser209 (eIF4ES209A), rendering the protein non-phosphorylatable by its upstream kinase, mitogen activated protein kinase interacting kinase (MNK), fail to demonstrate hyperalgesic priming to a range of stimuli including nerve growth factor (NGF) and proteinase activated receptor type 2 (PAR2) agonists. We therefore tested the hypothesis that eIF4E phosphorylation regulates BNDF translation. eIF4ES209A mice show no differences in expression of a wide variety of cell population markers in the dorsal root ganglion (DRG) or spinal cord compared to wild-type littermates. On the other hand, while BDNF transcription was similar for both genotypes, BDNF mRNAs were associated with lighter polysomes and BDNF protein was markedly reduced in eIF4ES209A DRG neurons, indicating that inhibition of eIF4E phosphorylation reduces the translation of BDNF mRNA. Importantly, although hyperalgesic priming to NGF and PAR2 agonists is strongly decreased or absent in eIF4ES209A mice, bypassing afferent-evoked release of BDNF with direct spinal injection of BDNF produces robust hyperalgesic priming in eIF4ES209A and wild-type mice. Our findings demonstrate that eIF4E phosphorylation plays a crucial role in BDNF translation and pathological pain plasticity following injury. Therefore, targeting eIF4E phosphorylation via the ERK/MNK pathway is a novel target for the manipulation of BDNF translation and pain plasticity.

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Poster

704. Peripheral Mechanisms: Pain and Touch

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Support: NINDS K24NS059892

NIH R01NS093653

Title: Analyzing axons within normal human skin biopsies reveals demographic differences and developmental pruning until the mid-20's

Authors: *A. L. OAKLANDER, H. M. DOWNS, M. M. KLEIN;
Dept Neurol., Mass Gen Hosp, Harvard Med. Sch., Boston, MA

Abstract: Background: “Small-fibers” are the unmyelinated C fibers and thinly myelinated Aδ PNS neurons that regulate body homeostasis and injury responses including pain sensation. Small-fiber polyneuropathies (SFPN) cause somatosensory (eg chronic pain, itch) and dysautonomic symptoms. Physicians diagnose SFPN with tiny skin biopsies from patients’ lower legs. These are immunolabeled to permit counting epidermal nerve fibers (ENF), which are predominantly small-fibers. Each patient’s ENF density is then compared to a distribution curve modeled from biopsying normal volunteers, and those with densities ≤ 5^{th} centile are diagnosed and treated for SFPN. These normative skin biopsies can also inform about normal neuroanatomy. **Methods:** With IRB permission, we biopsied distal-leg skin of 373 normal volunteers aged 8-92 years. They had been screened to exclude any with possible neuropathy, and adults had glucose-tolerance tests to exclude any with diabetes or pre-diabetes. Biopsies were vertically sectioned (50μm), PGP9.5-immunolabeled (Chemicon), and had ENF density measured by standard methods. 20 subjects had 2nd biopsies later for longitudinal study. **Results:** The 102 subjects younger than age 24 had far denser innervation than older adults (426 vs. 227 ENF/mm^2; $p<0.001$). Females had denser innervation than males (314 vs. 247/mm^2; $p<0.001$) and values from Asians were higher than from age-matched non-Asians (336 vs. 237/mm^2; $p<0.001$). Among the 13 subjects with repeat biopsies before age 24, mean density dropped by 46 ENF/mm^2/year, whereas in older subjects (n=9) losses slowed, averaging 13 ENF/mm^2/year. So we developed and use a multivariate normative model that incorporates age, gender, and race. We compared its diagnostic performance to the single normative threshold that most labs use (3.8 ENF/mm or 76 ENF/mm^2). In 2012-2013 our multivariate model identified ENF densities ≤ 5^{th} centile in 105 biopsies from patients under 41 years. In contrast, the single-threshold model only identified 26 among them (25%) as below the 5^{th} centile cutoff. Thus, labs that use this threshold may have a 75% false-negative rate that could hinder some SFPN patients from being diagnosed and treated. **Conclusions:** Skin-biopsy diagnosis of SFPN depends critically on having accurate norms for comparison. Adding demographic data into normative models improves diagnostic sensitivity, especially for younger, female, Asian patients. Evidence from both cross-sectional and longitudinal analyses suggest that children have superabundant epidermal innervation that is drastically pruned until the mid-20’s and then slowly declines throughout life, as occurs in the brain’s cortex.

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Poster

705. Pain Models: Pharmacology

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WSU College of Arts and Sciences

WSU Honors College

Title: Hyperbaric oxygen produces antinociception in mice by activating CB1 cannabinoid receptors

Authors: *A. C. STOUDT[1,2], Y. ZHANG[1], D. Y. SHIRACHI[3], R. M. QUOCK[2,1];
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Abstract: Exposure to increasing pressures of hyperbaric oxygen (HBO_2) produced a pressure-related antinociceptive response (Liu *et al.*, *Life Sci* 98:44-48, 2014). HBO_2 produces an acute antinociceptive effect in mice that is only partly antagonized by opioid receptor blockers (Heeman *et al., Brain Res* 1540:42-47, 2013). This study was conducted in order to determine whether HBO_2-induced antinociception might owe part of its effect to endocannabinoid mechanisms, which are also known to be involved in antinociception (Pertwee *et al.*, *Prog Neurobiol* 63:569-611, 2001). The antinociceptive responsiveness of male NIH Swiss mice to HBO_2 was assessed using the acetic acid abdominal constriction test. Different groups of mice were pretreated with the cannabinoid receptor type 1 (CB_1) antagonist/inverse agonist 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide (AM 251); and the anandamine transport-inhibitor N-(4-hydroxyphenyl)-5Z,8Z,11Z,14Z-eicosatetraenamide (AM 404); or their respective vehicles. Pretreatment with AM 251 caused a dose-dependent reduction in the magnitude of the antinociceptive response. Pretreatment with AM 404, which increases the availability of anandamide, enhanced the antinociceptive response to HBO_2. These findings are consistent with the hypothesis that endocannabinoid systems may contribute at least in part to HBO_2-induced antinociception.

Disclosures: **A.C. Stoudt:** None. **Y. Zhang:** None. **D.Y. Shirachi:** None. **R.M. Quock:** None.

Poster

705. Pain Models: Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 705.02/R3

Topic: D.08. Pain

Title: ALGOGram™: a new research tool to valuate drugs in multiple pain areas

Authors: Y. DARBAKY, *L. DIOP;
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Abstract: To evaluate the efficacy of exploratory compounds in different pain areas, various classical preclinical models are routinely used. For early drug discovery purposes, however, the full characterization package can be long and significantly expensive. To address this issue, we have developed an innovative screening tool, the ALGOGram™, a panel of behavioral pain models each validated with the most clinically relevant drugs. **Material and methods:** The ALGOGram™ is a battery of 11 validated animal models/tests spanning a broad range of pain areas (acute and tonic pain, neuropathic pain, inflammatory pain, post-operative pain, and visceral pain). The concept is an assessment of efficacy based on a group size of n=4 rats/model/test, thus providing a general pharmacological profile while reducing costs; assays/test are run in parallel, thus minimizing timelines. To validate the ALGOGram™, various reference drugs classically used in clinical pain practice (morphine, gabapentin, buprenorphine, tramadol, acetaminophen and diclofenac) were evaluated in the 11 pain models / tests (models: CCI, oxaliplatin, carrageenan, kaolin, post-operative and TNBS; tests: paw pressure, tail flick, writhing and formalin). Behavioral and acute toxicity were also evaluated (modified Irwin grid). Results are expressed for each group as a percentage of activity for each model/test calculated from the mean value of the vehicle-treated animals from our 9 year-historical database. **Results:** Buprenorphine, morphine and tramadol were active in all 11 different pain models. In contrast, gabapentin was active in several hypersensitive pain models. Diclofenac and acetaminophen displayed antinociceptive properties in some inflammatory pain models . Importantly, analgesic profiles obtained with n=4 animals in the ALGOGram™ were in line with those generated in various and repeated fully-powered studies as well as those described in the literature. **Conclusion:** The ALGOGram™ provides a rapid and predictive evaluation of investigational compounds in 11 different pain models/tests, enabling their prioritization for fully-powered studies. Shortened timelines and reduced costs are possible due to small group sizes that are run largely in parallel. In summary, the ALGOGram™ may prove to be useful in a signal detection exercise for a broad range of potential analgesic activity.

Disclosures: Y. Darbaky: A. Employment/Salary (full or part-time):; ANS Biotech. **L. Diop:** A. Employment/Salary (full or part-time):; ANS Biotech.

Poster

705. Pain Models: Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 705.03/R4

Topic: D.08. Pain

Title: The formalin test: initial excitation and biphasic activation

Authors: K. KISTNER[1], T. HOFFMANN[1], S. SAUER[1], T. KICHKO[1], P. REEH[1], *M. J. FISCHER[2];
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Abstract: In the formalin test, formaldehyde causes a characteristic biphasic pain behavior in rodents. We have recently hypothesized a mechanism explaining the occurrence of two phases. Initial excitation is rapidly followed by hyperpolarization due to a sodium current inhibition which silences primary afferents. Redistribution of formaldehyde recruits previously unexposed areas which leads to a secondary activation which is later terminated by further redistribution. Upon exposure to formaldehyde 30mM compound action potentials of nociceptive C-fibers showed a progressive increase in latency and decrease in amplitude, leading to complete and partially reversible conduction block. A-fibers showed slightly less sensitivity to formaldehyde but no recovery of conduction block. The mechanism of the initial activation phase by formaldehyde was so far unclear. The chemoreceptor channel TRPA1 was suggested as primary transducer, but the high concentrations used in the formalin test elicited a similar response in both TRPA1 wildtype and knockout animals. The present study addresses the cellular mechanism of the excitatory action of formaldehyde. We show via release of the neuropeptide CGRP from intact tissue that formaldehyde 40 mM causes a considerable activation in TRPA1 knockout animals. Formaldehyde 40 mM evokes a dose-dependent increase in cytosolic calcium. This calcium release stems from intracellular stores, as is present in the absence of extracellular calcium. This response and can be eliminated by depletion of intracellular calcium stores in the endoplasmic reticulum but not by depletion of mitochondrial calcium stores. The described mechanism was observed in all cells tested, including mouse sensory neurons and primary keratinocytes, but also in non-neuronal cell lines, and is therefore independent of TRPA1. Inhibition of the sarco/endoplasmic reticulum calcium-ATPase had a major contribution. Many cellular excitability pathways are calcium dependent. We thus hypothesize that the above TRPA1-independent mechanism may underlie formaldehyde-induced pan-neuronal excitation and subsequent inflammation in the peripheral nociceptive system.

Disclosures: K. Kistner: None. **T. Hoffmann:** None. **S. Sauer:** None. **T. Kichko:** None. **P. Reeh:** None. **M.J. Fischer:** None.

Poster

705. Pain Models: Pharmacology

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Program#/Poster#: 705.04/R5

Topic: D.08. Pain

Support: NIH Grant K08GM102691

Title: AMPAKines relieve both pain and pain-related depression by potentiating AMPA receptors in the nucleus accumbens

Authors: *C. SU[1], H. LIN[1], N. PAWLAK[2], J. WANG[1];
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Abstract: Background: Pain and its associated comorbidities such as depression are a serious public health problem. Novel analgesic options that do not suppress the respiratory drive are urgently needed. AMPAkines potentiate AMPA receptor signaling and have been shown to oppose sedative-induced respiratory depression. Our previous study has shown that systemic administration of an AMPAkine, CX546, can relieve both sensory and depressive symptoms of chronic neuropathic and inflammatory pain in rats. However, the following questions remain unanswered. 1) Can AMPAkines relieve sensory and affective symptoms of acute pain as well? 2) What is the mechanism for AMKAkine analgesia? Recent human imaging and animal studies suggest that glutamate signaling in the nucleus accumbens (NAc) plays a critical role in pain regulation. Thus, we hypothesized that AMPAkines can relieve acute and chronic pain by potentiating glutamate transmission through AMPA receptors in the NAc. Methods: We used paw incision (PI) and spared nerve injury (SNI) to model acute pain and chronic neuropathic pain in rats. CX546 was administered systemically or locally infused into the NAc in both pain models. We used mechanical allodynia to assay the effect of AMPAkines on sensory pain symptoms. We used the forced swim test (FST) to assess the effect of AMPAkines on the depressive symptoms of pain. To further verify the specificity of the NAc as a pharmacologic target for AMPAkines, we infused 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX), an antagonist of AMPA receptors, into the NAc to block the pain-relieving effects of systemic administration of CX546. Results: Compared with control, systemic administration of CX546 reduced sensory allodynia in the PI model. In addition, local infusion of CX546 into the NAc also reduced sensory allodynia. Furthermore, both systemic and local (intra-NAc) infusion of CX546 reduced depressive symptoms of acute pain, as shown by an improved performance on the FST test. Meanwhile, in the SNI model, local infusion of CX546 also reduced sensory and depressive symptoms of pain. Finally, we found that NBQX in the NAc blocked the effects of systemically administration of CX546 on mechanical allodynia and FST in both PI and SNI models. Conclusion: Our results demonstrate that CX546, an AMPAkine, can relieve both sensory and depressive symptoms of acute and chronic pain. Furthermore, the pain-

relieving property of the CX546 is likely mediated by its potentiating effect on AMPA receptors in the NAc. Thus, these drugs can be a novel class of analgesics for the treatment of both acute and chronic pain.

Disclosures: C. Su: None. **H. Lin:** None. **N. Pawlak:** None. **J. Wang:** None.

Poster

705. Pain Models: Pharmacology

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Topic: D.08. Pain

Support: NIH Grant DA016644

Title: Antinociceptive effects of JWH015, a synthetic cannabinoid

Authors: N. Z. GREENE, *R. M. CRAFT;
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Abstract: The primary psychoactive ingredient in marijuana, THC, is a cannabinoid that produces pain relief by acting at CB1 and CB2 receptors in the nervous system. However, THC's actions at CB1 receptors also result in unwanted side-effects such as sedation, anxiety, and feeling "high." Synthetic cannabinoids have been developed that have CB2 receptor selectivity, and these may be useful in the treatment of chronic pain while producing fewer unwanted side-effects than THC. While previous research has suggested that there are sex differences in THC's mechanism of action at CB1 and CB2 receptors, there is little data regarding potential sex differences in the mechanism of action of synthetic, CB2-preferring cannabinoids. Using the Complete Freund's Adjuvant (CFA) model of chronic inflammatory pain, the antinociceptive effects of the synthetic, CB2-preferring cannabinoid JWH015 were examined in rats of both sexes. Rats received an injection of CFA into one hind paw, creating a model of chronic inflammatory pain. Three days after CFA injection, rats received either vehicle or the CB2-preferring cannabinoid JWH015 (5 or 10 mg/kg), and pain tests were conducted at 15-240 minutes post-injection. In both sexes, JWH015 significantly decreased CFA-induced mechanical and thermal hypersensitivity, with no significant reduction in biased weight-bearing or in edema. In a subsequent experiment, cannabinoid antagonists were used to determine whether JWH015's antinociceptive effects are mediated by the CB1 and/or CB2 receptor. Three days after receiving CFA, vehicle, the CB1 receptor-selective antagonist rimonabant (1 mg/kg), or the CB2 receptor-selective antagonist SR144528 (1 mg/kg) was administered; 30 minutes later, vehicle or

JWH015 (10 mg/kg) was administered. Rimonabant and SR144528 each significantly reversed JWH015's effects in both sexes. This result indicates that JWH015's antinociceptive effects are mediated by both CB1 and CB2 receptors in both males and females. Therefore, despite its CB2-preferring binding profile, JWH015 may not provide any significant clinical advantage over THC in the treatment of chronic pain.

Disclosures: N.Z. Greene: None. **R.M. Craft:** None.

Poster

705. Pain Models: Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 705.06/R7

Topic: D.08. Pain

Support: JSPS KAKENHI 15K20377

Title: Mechanism underlying oral ulcerative mucositis-induced pain in chemotherapy-received rat model

Authors: K. YAMAGUCHI, *K. ONO, S. HITOMI, N. HARANO, T. SAGO, S. WATANABE, K. INENAGA;
Kyushu Dent. Univ., Fukuoka, Japan

Abstract: In many cancer patients, oral ulcerative mucositis is developed as a side effect of chemotherapy and causes severe pain. However, oral ulcerative mucositis-induced pain following chemotherapy has not been studied well. In this study, we investigated pain-related behaviors in oral ulcer model of rats with intraperitoneal administration of the chemotherapeutic drug, 5-fluorouracil (cumulative dose: 120 mg/kg). Using our recently-developed assay system for intraoral pain in conscious rats, spontaneous pain, evoked pain, and mechanical allodynia were evaluated from spontaneous mouth rubbing time, evoked mouth rubbing time after drop of stimulus-containing solution on oral mucosa, and head-withdrawal threshold to mechanical stimulation to the mucosa, respectively. The model showed long-term leukopenia, severe oral mucositis, and excessive bacterial colonization in the ulcer region compared with saline administration. Oral ulcer-induced spontaneous pain and mechanical allodynia were exaggerated following 5-fluorouracil administration. Antibacterial pretreatment suppressed the spontaneous pain largely and mechanical allodynia partially. The spontaneous pain was largely suppressed by indomethacin administration, but the mechanical allodynia was not changed. Dropping application of the TRPV1 agonist capsaicin in the oral mucosa transiently evoked mouth rubbing

and the behavior was further exaggerated by 5-fluorouracil. In 5-fluorouracil administrated oral ulcer model, administration of the TRPV1 antagonist SB366791 significantly inhibited not only the capsaicin-evoked pain, but also spontaneous pain. From these results, spontaneous and capsaicin-evoked pain in the 5-fluorouracil-administrated model are mainly mediated by infectious inflammation via TRPV1 activation. Mechanical allodynia is caused by mucosal tissue injury and/or bacterial toxins, based on sensitivity to antibacterial pretreatment and resistant to indomethacin.

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Poster

705. Pain Models: Pharmacology

Location: Hall A

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Program#/Poster#: 705.07/R8

Topic: D.08. Pain

Title: Anti nociceptive effects of carica papaya leaves extract: modulation by dopaminergic, gabaergic and serotonergic receptors

Authors: ***B. V. OWOYELE**[1], R. A. AWEDA[2], A. A. BABALOLA[2];
[1]Physiol., Univ. of Ilorin, Ilorin, Nigeria; [2]Physiol., Univ. of Ilorin, Ilorin, Nigeria

Abstract: Pain is the most common factor for patients visit to the clinics. However, the available drugs can not treat all the various types of pain without the manifestations of side effects. Therefore, there is increasing research on alternative sources of therapeutic agents for the treatment of pain. The anti-nociceptive and anti-inflammatory effects of Carica papaya extracts (CPE) have been reported in the literature but the mechanism of action has not been fully described. The aim of the present study therefore, is to investigate the possible role of dopaminergic gabaergic and serotonergic receptor mechanisms on the analgesic effects of CPE in Wistar rats using the antagonists bicuculline, phaclofen, risperidone and haloperidol. The animals were divided into seven groups based on the drugs/saline administered. The control group received saline and the reference group received indomethacin (10 mg/kg). The other five groups received CPE (150 mg/kg), bicuculline (2mg/kg) + CPE (150 mg/kg), phaclofen (1mg/kg) + CPE (150 mg/kg), risperidone (2mg/kg) + CPE (150 mg/kg), and haloperidol (1 mg/kg)+CPE (150 mg/kg). Anti nociceptive activities were accessed by tail immersion and formalin-induced paw licking models. The results showed that all the antagonists used enhanced the anti nociceptive effect of CPE in the thermal pain but the effects of bicuculline was more

pronounced. However, the antagonist only enhanced the antinociception in the late phase of the formalin induced pain. In conclusion, the analgesic effects of CPE is not via stimulation of dopaminergic, gabaergic and serotonergic receptors but blockage of these receptors can enhance the anti-nociceptive effect of CPE.

Disclosures: **B.V. Owoyele:** None. **R.A. Aweda:** None. **A.A. Babalola:** None.

Poster

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Topic: D.08. Pain

Support: JSPS KAKENHI 15K11272

MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2013-2017

Title: Oxytocin alleviates orofacial hypersensitivity following infraorbital nerve injury in rats

Authors: ***A. KUBO**[1], M. SHINODA[1], D. C. YEOMANS[2], K. IWATA[1];
[1]Dept. of Physiol., Nihon Univ. Sch. of Dent., Tokyo, Japan; [2]Fac. of Anesthesia, Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Oxytocin (OXT) is one of the neuropeptides synthesized and secreted from neurons in the paraventricular nucleus and supraoptic nucleus of hypothalamus. Many reports have suggested that OXT plays an important role in pain modulation and analgesia. However, the underlying mechanisms regarding anti-nociceptive effect on neuropathic pain are not fully understood. We examined the effect of OXT on trigeminal neuropathic pain associated with partial ligation of the infraorbital nerve in rats. The head-withdrawal threshold to mechanical stimulation (MHWT) of the maxillary whisker pad skin on the side ipsilateral to the ligation was measured using von Frey filaments. After confirming of the reduced MHWTs 5-8 days after nerve ligation (the mean of reduced MHWTs, 10.8 ± 5.3 g), MHWTs were measured 2, 5, 8 and 24 hrs after OXT (1 mM, 1 μl) administration into the trigeminal ganglion (TG) using the injection-cannula set. The reduced MHWTs were significantly recovered at 2 and 5 hrs after the OXT administration compared with that of vehicle (PBS) (2 hrs after: OXT group, 35.4 ± 4.2 g; PBS group, 10.8 ± 3.9 g; $p < 0.01$; 5 hrs after: OXT group, 35.6 ± 10.9 g; PBS group, 14.8 ± 5.2; $p < 0.05$; $n = 5$ in each group). This effect did not sustain until 8 hrs after and completely

returned to the pre-administration level 24 hrs after the OXT administration. We also examined the change in the excitability of TG neurons acutely isolated from the neuropathic pain model rats. Ten μM of OXT was applied in the culture medium 2-5.5 hrs before patch-clamp recording. The resting membrane potentials of OXT-treated TG neurons were significantly decreased (OXT group, -63.4 ± 1.1 mV; PBS group, -56.2 ± 2.2 mV; $p < 0.01$; $n = 12$ in each group). Threshold currents in OXT-treated neurons for spike generation during current injection were also significantly greater than that of PBS (OXT group, 163.3 ± 27.6 pA; PBS group, 89.2 ± 12.4 pA; $p < 0.05$; $n = 12$ in each group). Present findings suggest that OXT could be at least partially effective on the suppression of hyperexcitability of TG neurons and exert analgesia on orofacial neuropathic pain.

Disclosures: **A. Kubo:** None. **M. Shinoda:** None. **D.C. Yeomans:** None. **K. Iwata:** None.

Poster

705. Pain Models: Pharmacology

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Topic: D.08. Pain

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Title: Novel methylcyclohexyl benzene derivatives: functional cannabinoid receptor mediated antinociception vs cannabimimetic effects in mice

Authors: *J. L. WILKERSON[1], T. GRIM[1], A. MORALES[1], M. BHOWMICK[2], A. MAHADEVAN[2], A. H. LICHTMAN[1];
[1]Dept. of Pharmacol. & Toxicology, Virginia Commonwealth Univ. MCV, Richmond, VA;
[2]Organix Inc., Woburn, MA

Abstract: Aims: Therapeutic options that lack potential abuse liability for the treatment of pathological pain are desperately needed. Novel methylcyclohexyl benzene derivatives have been synthesized for the intended therapeutic development of new analgesics that may activate cannabinoid receptors. Activation of the cannabinoid receptors, either the cannabinoid 1 receptor

predominately found on neurons (CB1R) or the cannabinoid 2 receptor (CB2R), predominately found on immune cells, have shown promise in preclinical animal models for pain control. However, activation of the CB1R leads to cannabimimetic effects, and is undesirable for pain therapeutics. Here we sought to characterize the antinociceptive effects of novel methylcyclohexyl benzene derivatives, determine the degree to which CB1R and/or CB2R mediates these effects, and examine common cannabimimetic side effects that would dampen enthusiasm for clinical development. Methods: The chronic constriction injury (CCI) of the sciatic nerve model of neuropathic pain or sham surgeries were performed in male ICR mice. CCI produces robust increases in sensitivity to light mechanical touch, or allodynia, as assayed with the von Frey test, and increased thermal sensitivity, or thermal hyperalgesia, as assayed with the hotplate test. Cannabimimetic effects were inferred by the occurrence of hypothermia, catalepsy, and antinociception in ICR mice. The dose response of each drug was assessed in each assay, and all compounds were administered intraperitoneally. Results: The novel methylcyclohexyl benzene derivatives O-9598, O-9599, and O-9603 produced dose-dependent and time-dependent reversal of allodynia and thermal hyperalgesia, with the calculated ED50 for anti-allodynic effects being 0.54, 12.43, and 53.17 mg/kg, respectively. The anti-thermal hyperalgesia ED50 for O-9598, O-9599 and O-9603 were calculated to be 3.48, 15.02, and 51.59 mg/kg, respectively. The CB1R antagonist rimonabant, but not the CB2R antagonist SR144528, fully blocked in both ipsilateral and contralateral paws the anti-allodynic and anti-thermal hyperalgesic effects of O-9598 and O-9602. Interestingly, whereas in the ipsilateral paw, the anti-allodynic effects of O-9599 were blocked by rimonabant, SR144528 blocked the anti-allodynia effects in the contralateral paw and in the hotplate test. Finally, O-9598, but not O-9599 or O-9603 produced significant cannabimimetic effects. Conclusions: Novel methylcyclohexyl benzene derivatives show efficacy in the CCI preclinical model of neuropathic pain. Out of the compounds assessed thus far, O-9599 displays the best profile as a potential lead compound.

Disclosures: **J.L. Wilkerson:** None. **T. Grim:** None. **A. Morales:** None. **M. Bhowmick:** A. Employment/Salary (full or part-time):; Organix Inc. **A. Mahadevan:** A. Employment/Salary (full or part-time):; Organix Inc.. **A.H. Lichtman:** None.

Poster

705. Pain Models: Pharmacology

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Program#/Poster#: 705.10/R11

Topic: D.08. Pain

Support: Grants-in-Aid and by special coordination funds from Grants-in-Aid for Scientific Research (C) (25462458) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Influence of GPR40/FFA1 on the regulation of central post-stroke pain

Authors: *S. TOKUYAMA, S. HARADA, K. NAKAMOTO;
Kobe Gakuin Univ., Kobe, Japan

Abstract: Central post-stroke pain (CPSP) is one of the complications of cerebral ischemia and neuropathic pain syndrome. In this study, we assessed the role of GPR40/FFA1, a long-chain fatty acid receptor, showing anti-nociceptive effects mediated by the release of β-endorphin, in CPSP. We also examined the role of astrocytes in CPSP due to their effects in mediating the release of polyunsaturated fatty acids. The aim of this study was to determine the interactions between CPSP and astrocyte/GPR40 signaling. Male ddY mice were subjected to 30 min of bilateral carotid artery occlusion (BCAO). The development of hind paw mechanical hyperalgesia was measured using the von Frey test. Neuronal damage was estimated by histological analysis on day 3 after BCAO. Analysis of free fatty acids (FFAs) in the mouse hypothalamus used LC-ESI-MS/MS methods. The thresholds for hind paw mechanical hyperalgesia were significantly decreased on days 1-28 after BCAO when compared with pre-BCAO assessments. BCAO-induced mechanical hyperalgesia was significantly decreased by intracerebroventricular injection of docosahexaenoic acid or GW9508, a GPR40/FFA1 agonist; furthermore, these effects were reversed by GW1100, a GPR40/FFA1 antagonist. The expression levels of glial fibrillary acidic protein, an astrocytic marker, were significantly decreased 5 h after BCAO, but not hypothalamic GPR40/FFA1 protein expression. At 5 hr after BCAO, some FFAs (palmitate, stearate, oleinic acid, linoleic acid, arachidonic acid and DHA) were significantly decreased as compared with sham group. These results suggest that BCAO-induced mechanical hyperalgesia can be regulated by astrocyte activation and stimulation of GPR40/FFA1 signaling.

Disclosures: **S. Tokuyama:** None. **S. Harada:** None. **K. Nakamoto:** None.

Poster

705. Pain Models: Pharmacology

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Program#/Poster#: 705.11/R12

Topic: D.08. Pain

Title: Olfactory bulb evoked field potential by electrical stimulation of the olfactory epithelium in the anesthetised mouse: development of a potential Nav1.7 channel blocker assay

Authors: *J. ALLARD, W. MINER;
E-Phys, Clermont-Ferrand, France

Abstract: The voltage gated sodium channel 1.7 (Nav1.7) is mandatory for the transmission of action potentials from olfactory sensory neurons to their post-synaptic target in the olfactory bulb (OB). Thus, evoked field potential (EFP) in the OB to electrical stimulation of the olfactory epithelium (i.e. orthodromic OB EFP) should constitute a useful *in vivo* Nav1.7 channel blocker assay. The aim of this work was to set up such an assay in anaesthetised mice. Antidromic EFP generated by stimulation of the lateral olfactory tract (LOT) was performed as a control. C57BL/6J mice were anesthetised with urethane. The animal's head was placed in a stereotaxic frame. Holes were made above the parietal, frontal and nasal bones for access to the LOT, the OB or the nasal cavity, respectively. Electrical stimulation of the LOT and olfactory epithelium was conducted with bipolar concentric and paired platinum wire electrodes, respectively. A single parylene-coated tungsten electrode was used for recording. EFP were recorded at different depths in the OB. Stimulus intensity-response curves were constructed via recordings in the granule cell layer. Local application of lidocaine, CoCl2 and tetrodotoxin (TTX) at the surface of the site of recording, as well as neuromuscular blockade, were used to assess the specificity of the recorded EFP. The shape of the EFP was depth dependent. In the granule cell layer, both orthodromic and antidromic EFP were mainly characterized by a large positive deflection, with an average latency of 10.7 and 2.4 ms respectively, followed by a negative deflection. The amplitude of the positive deflection was approximately 5 times greater, and its duration 3 times shorter with antidromic stimulation compared with orthodromic stimulation. The negative deflection was also much larger (amplitude and duration) with antidromic stimulation. Stimulus intensity-responses of orthodromic EFP were right shifted compared to antidromic EFP. The area under the curve of the rectified orthodromic EFP was decreased by about 80% when 2% lidocaine was applied at the recording site, and by 40% when CoCl2 10 mM was used. It was virtually abolished when TTX was applied at 10 μM. In contrast, antidromic EFP was barely affected by local application of CoCl2 at the recording site, and less sensitive to the inhibitory effect of lidocaine. Neuromuscular blockade did not change EFP. The shape, latency and lidocaine sensitivity of the orthodromic OB EFP recorded suggest that the signal measured was not an artefact. Nevertheless, the high concentration of TTX necessary to abolish the response, and the partial sensitivity to CoCl2 requires further investigation to ensure specificity of the assay.

Disclosures: J. Allard: None. **W. Miner:** None.

Poster

705. Pain Models: Pharmacology

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Program#/Poster#: 705.12/R13

Topic: D.08. Pain

Title: A comparison between nonhuman primate and rat models of acute chemotherapy-induced peripheral neuropathy

Authors: Y. SHIDAHARA[1], S. NEMOTO[1], Y. AWAGA[1], M. TAKASHIMA[1], *A. HAMA[1], A. MATSUDA[1], H. TAKAMATSU[1], K. UMEMURA[2];
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Abstract: Oxaliplatin is a key platinum-based chemotherapeutic used in the treatment of colorectal cancer. Unique to other chemotherapeutics, oxaliplatin causes an acute cold hypersensitivity in the extremities which appears within hours or days after infusion. This peripheral neuropathy is disabling and is often a reason to limit or terminate treatment. Thus, there is a need for treatments that alleviate neuropathic symptoms so that fully efficacious oxaliplatin treatment can be utilized. There is currently a lack of treatments for oxaliplatin-induced peripheral neuropathy. The lack of treatments is due in part to a lack of confidence of the predictiveness of preclinical rodent models of chemotherapy-induced peripheral neuropathy. Thus, a nonhuman primate (NHP) model of oxaliplatin-induced peripheral neuropathy was developed and a comparison of the effects of clinical analgesics, between the NHP model and a rat model of oxaliplatin-induced peripheral neuropathy, was performed. In NHP, sensitivity of the feet to pressure was assessed using a hand-held pressure meter. In rats, sensitivity to cold was assessed by application of acetone to the hind paw and in NHP, tail withdrawal latency (in seconds) to cold water (10 oC) was measured. In oxaliplatin-treated rats, increased responding to acetone (“cold allodynia”) was observed 17 days after the first dose of oxaliplatin. In NHPs, a significant decrease in withdrawal latency to cold water (“cold allodynia”) was observed beginning 3 days after oxaliplatin infusion. In oxaliplatin-treated NHP, no significant change was observed in response to pressure. Pregabalin (30 mg/kg, p.o.), tramadol (30 mg/kg, p.o.) and duloxetine (30 mg/kg, p.o.) ameliorated oxaliplatin-induced cold allodynia in rats. While duloxetine (30 mg/kg, p.o.) ameliorated oxaliplatin-induced cold allodynia in NHP, pregabalin (30 mg/kg, p.o.) and tramadol (30 mg/kg p.o.) had no effect on cold allodynia. These results indicate that pain-related behavior seen in NHP is similar to that observed in clinical oxaliplatin-induced peripheral neuropathy. Furthermore, the efficacy of the drugs in the NHP model mirrors the efficacy observed clinically. The current NHP model could be highly useful in testing novel treatments for oxaliplatin-induced peripheral neuropathy.

Disclosures: **Y. Shidahara:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc. **S. Nemoto:** 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. **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

705. Pain Models: Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 705.13/R14

Topic: D.08. Pain

Support: Arthritis Research UK (Grant number 20020)

Title: Microglia contribute to non-inflammatory pain in early phases of collagen-induced arthritis

Authors: F. R. NIETO[1], A. K. CLARK[1], J. GRIST[1], V. CHAPMAN[2], *M. A. MALCANGIO[3]; [1]The Wolfson Ctr. for Age-Related Dis., King's Col. London, London, United Kingdom; [2]Arthritis Res. UK Pain Centre, Sch. of Life Sci., Univ. of Nottingham, Nottingham, United Kingdom; [3]Wolfson CARD, The Wolfson Wing, London, United Kingdom

Abstract: Despite pain being a dominant feature of rheumatoid arthritis (RA), our understanding of RA pain mechanisms is modest. Specifically, the weak correlation between pain and swelling in patients, suggests that mechanisms other than overt inflammation contribute to pain in RA. This clinical observation is mirrored in the early development phase of collagen-induced arthritis (CIA) in the rat, in which mechanical hypersensitivity develops before inflammation in the hind paw. We hypothesised that in the CIA model of RA the early non-inflammatory pain behaviour is due to activation of nociceptors and spinal sensitization mechanisms, including a significant microglia response. Thus, we evaluated the extent of a microglial response in the dorsal horn and hind paw mechanical hypersensitivity in the absence and presence of swelling. Then, we quantified cyto(chemo)kine levels in the CSF as candidate pro-nociceptive mediators released by activated microglia. Finally, we tested the effect of microglial inhibitors on the development of pain as well as spinal microgliosis and cyto(chemo)kine levels in the cerebrospinal fluid (CSF). We observed that CIA rats, but not control rats, displayed significant microgliosis (increased

number of Iba1 p-p38 positive cells) in the dorsal horn from day 7 (before the onset of signs of arthritis e.g. swelling) up to day 18 post-immunization. At this time interval from immunization microgliosis had reached its highest levels which coincided with mechanical hypersensitivity and severe swelling in the hind paws. The levels of IL-1β in CSF of 7 day-CIA rats were significantly higher than in controls and just detectable in plasma (Multiplex assay). Similarly, FKN levels in CSF of 13 day-CIA rats were higher than in controls (ELISA). These data suggest that there is a central source for these cyto(chemo)kines and microglia are likely to contribute to the release and effect of these pro-nociceptive mediators. Consistently, intrathecal delivery of the microglial inhibitor LHVS (from 24 h prior to 13 days after immunization) attenuated i) development of mechanical hypersensitivity, ii) spinal microglial response and iii) increment of FKN in the CSF. In addition the delivery of a FKN-neutralising antibody (from 24 h prior to 13 days after immunization) attenuated the development of mechanical hypersensitivity and spinal microglial response in CIA rats. However neither treatment affected hind paw swelling. Altogether these data suggest that microglia play a key role in the central mechanisms of non-inflammatory pain in arthritis. Thus, the inhibition of microglia-driven mechanisms provides opportunities for the treatment of this early pain.

Disclosures: F.R. Nieto: None. **A.K. Clark:** None. **J. Grist:** None. **V. Chapman:** None. **M.A. Malcangio:** None.

Poster

705. Pain Models: Pharmacology

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Topic: D.08. Pain

Support: Grants-in-Aid for Scientific Research 26870451

Grants-in-Aid for Scientific Research 26860384

Grants-in-Aid for Scientific Research 24591684

Title: [Leu11]-HK-1-derived peptides with D-Trp have longer antipruriceptive effects in rats

Authors: H. FUNAHASHI[1], R. NAONO-NAKAYAMA[2], Y. MIYAHARA[1], *Y. ISHIDA[1], T. NISHIMORI[1];
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Abstract: Hemokinin-1 (HK-1) is a mammalian tachykinin peptide consisting of 11 amino acids. Recently, it was demonstrated that the pretreatment with [Leu11]-HK-1, in which Met at the C-terminal of HK-1 was replaced by Leu, attenuated scratching induced by intrathecal administration of HK-1. In addition, the pretreatment with [Leu11]-HK-1 reduced the induction of scratching behavior by pruritogens such as histamine and serotonin, suggesting that [Leu11]-HK-1 may have an inhibitory effect on itch processing. Furthermore, it is believed that replacement of amino acids by D-tryptophan (D-Trp), prolongs the duration of effective time. Therefore, to clarify the duration of effective time of [Leu11]-HK-1-derived peptides, the effect of pretreatment with [D-Trp7]-[Leu11]-HK-1, [D-Trp9]-[Leu11]-HK-1 and [D-Trp7,9]-[Leu11]-HK-1 on the induction of scratching behavior by the intrathecal administration of HK-1 and by the intradermal injection of chloroquine was evaluated. The induction of scratching by intrathecal administration of HK-1 was significantly suppressed until 24 hours after pretreatment with [D-Trp7]-[Leu11]-HK-1 and [D-Trp9]-[Leu11]-HK-1 and 4 hours after [D-Trp7,9]-[Leu11]-HK-1 treatment. On the other hand, intrathecal administration of [D-Trp7,9]-[Leu11]-HK-1 and [D-Trp9]-[Leu11]-HK-1 similarly inhibited the induction of scratching behavior by intradermal administration of a pruritogen, chloroquine. Taken together, these results suggest that the antipruriceptive effects of [Leu11]-HK-1-derived peptides replaced by D-Trp may be unrelated to the number of D-Trp.

Disclosures: H. Funahashi: 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.; Grants-in-Aid for Scientific Research 26870451. **R. Naono-Nakayama:** None. **Y. Miyahara:** None. **Y. Ishida:** None. **T. Nishimori:** None.

Poster

705. Pain Models: Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 705.15/R16

Topic: D.08. Pain

Support: 261836

Title: Anti-allodynic effect of a sigma-1 antagonist in a model of neuropathic pain in rats

Authors: J. V. ESPINOSA-JUAREZ[1], O. A. JARAMILLO-MORALES[1], J. N. CORONA-RAMOS[1], J. G. NAVARRETE-VÁZQUEZ[2], *F. J. LOPEZ MUNOZ[1];
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Abstract: BACKGROUND. The treatment of neuropathic pain is performed according to the etiology, the most used drugs are neuromodulators, however exist others that may be potentially useful. The aim of this study was to compare the anti-allodynic effects (efficacy and potency) of a sigma-1 antagonist (BD-1063) and gabapentin using a model of neuropathic pain. METHODS: Wistar male rats were employed and subjected to chronic constriction injury (CCI), 10 days after surgery the anti-allodynic effect (acetone test) after single-dose of gabapentin (3.2-177.8 mg/kg p.o) and BD-1063 (5.6-56.2 mg/kg s.c.) were tested. RESULTS: In all cases the anti-allodynic effects increased in a dose-dependent manner. The time-course analysis shows that gabapentin (100 mg/kg) reached its maximum effect at 30 min after the treatment, producing an anti-allodynic effect of 65.5 ± 6.9 %, whereas BD-1063 (56.2 mg/kg) produced their maximum effect at 30 min with 90.8 ± 2.7 %; these anti-allodynic effects remained during 180 min of observation. Analyzing dose-response curves, BD-1063 exhibited similar efficacy to gabapentin. For its part regarding the analysis of pharmacological potency, we compare the ED50, BD-1063 antagonist showed higher potency than gabapentin. CONCLUSION: These results suggest that the antagonist of the sigma-1 receptor could be as effective as the reference drug gabapentin (similar efficacy and Emax) in the treatment of neuropathic pain. But the anti-allodynic effect could be achieved with lower doses of the sigma-1 antagonist (because its greater anti-allodynic potency).

Disclosures: **J.V. Espinosa-Juarez:** None. **O.A. Jaramillo-Morales:** None. **J.N. Corona-Ramos:** None. **J.G. Navarrete-Vázquez:** None. **F.J. Lopez Munoz:** None.

Poster

705. Pain Models: Pharmacology

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: NIH Grant AT007222

WSU College of Arts and Sciences

WSU Honors College

Title: Investigating the antinociceptive effect of hyperbaric oxygen in an animal model of fibromyalgia: role of nitric oxide

Authors: *P. SMITH[1], A. L. BREWER[1], Y. ZHANG[2], D. Y. SHIRACHI[3], R. M. QUOCK[1]; [1]Psychology, [2]Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA; [3]Physiol. and Pharmacol., Univ. of the Pacific, Stockton, CA

Abstract: Hyperbaric oxygen (HBO_2) has been shown to produce antinociception in acute, chronic, and inflammatory pain models in animals [Chung *et al., J Pain,* 11:847-853, 2010; Gibbons *et al.*, *Brain Res.* 1537:111-116, 2013; Sümen *et al., Eur J Pharmacol* 431:265-268, 2001]. This antinociceptive effect of HBO_2 has been shown to last for up to two weeks in both chronic and acute pain models [Gibbons *et al.*, *ibid.*; Chung *et al., ibid.*]. The antinociceptive effect of HBO_2 is mediated by nitric oxide (NO) according to previous studies [Chung *et al., ibid.*]. It has been reported that HBO_2 is effective in reducing pain in human subjects suffering from fibromyalgia [Yildiz *et al., J Int Med Res* 32:263-267*,* 2004]. The mechanism of HBO_2-induced antinociception in fibromyalgia has not been investigated to our knowledge. The acidic saline model is a rodent model of fibromyalgia and has been shown to mimic some of the symptoms of the disease [Sluka *et al., Muscle Nerve* 24:37-46*,* 2001]. The aim of this work was to determine if treatment with HBO_2 treatment was effective in reducing nociception in an animal model of fibromyalgia and ascertain whether NO mediates the antinociceptive effect of HBO_2. Baseline paw pressure measures were taken, using a Randall-Selitto paw pressure analgesy-meter (Ugo Basile). Animals were injected unilaterally in the gastrocnemius muscle with 20 µl pH 4 acidic saline or pH 7 saline (vehicle). Two days later, animals were injected again with either acidic saline or saline. After 90 min recovery, animals were exposed to a single 60-min treatment of HBO_2 at 3.5 ATA or exposed to room air. After another 90 min, mice were assessed, using the paw pressure assay. Results indicate that a single 60-min treatment of HBO_2 significantly increased paw pinch thresholds compared to animals exposed only to the acidic saline model of fibromyalgia. Other groups of mice were injected s.c. with either L-N^G-nitro-arginine (L-NOARG) or vehicle 60 min before pain testing. We showed that the HBO^2-induced antinociceptive effect was reversed by inhibition of nitric oxide synthase. These data indicate that HBO_2 effectively reduces nociception caused in the acidic saline model and that the antinociceptive effect of HBO_2 may be mediated by production of nitric oxide.

Disclosures: P. Smith: None. **A.L. Brewer:** None. **Y. Zhang:** None. **D.Y. Shirachi:** None. **R.M. Quock:** None.

Poster

705. Pain Models: Pharmacology

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Topic: D.08. Pain

Support: NIH Grant AT007222

WSU College of Arts and Sciences

WSU Honors College

Title: Hyperbaric oxygen suppresses paclitaxel-induced neuropathic pain through the rostral ventromedial medulla (RVM)

Authors: Y. ZHANG[1], D. Y. SHIRACHI[3], *R. M. QUOCK[2];
[1]Integrative Physiol. and Neurosci., [2]Psychology, Washington State Univ., Pullman, WA;
[3]Physiol. and Pharmacol., Univ. of the Pacific, Stockton, CA

Abstract: Hyperbaric oxygen (HBO_2) treatment can produce antinociception in rats with various neuropathic pain conditions [Thompson *et al.*, *Neurosci Res* 66:279-283, 2010; Gibbons *et al.*, *Brain Res* 1537:111-116, 2013]. Recently we reported that HBO_2 suppressed paclitaxel-induced neuropathic pain via a supraspinal mechanism in rats [Zhang *et al.*, *4th Int Cong Neuropathic Pain*, 2013]. It is known that the rostral ventromedial medulla (RVM) is the output of the pain-modulating system in the brain, and sends descending signals to the dorsal horn to modulate nociception. The aim of this study is to examine whether the RVM is involved in the pathway by which HBO_2 suppresses neuropathic pain. Male Sprague Dawley rats developed neuropathic pain following four repeated injections of 1.0 mg/kg paclitaxel, which was assessed by both mechanical and cold allodynia. After the last injection of paclitaxel, the rats received a single or four daily 60-min treatments with HBO_2 at 3.5 atmospheres absolute. To test involvement of the RVM, 2% w/v lidocaine in 0.5 μL saline was delivered into the RVM by microinjection immediately before HBO_2 treatment [Saadé *et al.*, *Pain* 149:89-99, 2010]. The assessment of allodynic response was carried out every other day and lasted for 32 days after the first paclitaxel injection. Results showed that a single HBO_2 treatment suppressed paclitaxel-induced mechanical allodynia, but not cold allodynia. Four repeated HBO_2 treatments reduced the cold allodynia. However, the antinociceptive effect of HBO_2 on mechanical allodynia was blocked by the microinjection of lidocaine into the RVM. This demonstrated that HBO_2 indeed suppressed paclitaxel-induced neuropathic pain via the RVM.

Disclosures: Y. Zhang: None. **D.Y. Shirachi:** None. **R.M. Quock:** None.

Poster

705. Pain Models: Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 705.18/R19

Topic: D.08. Pain

Title: Broad spectrum efficacy in rodent pre-clinical pain models with LY2969822, a metabotropic glutamate2/3 agonist prodrug

Authors: *M. P. JOHNSON[1], M. A. MUHLHAUSER[1], E. S. NISENBAUM[1], R. M. A. SIMMONS[1], B. M. FORSTER[1], K. L. KNOPP[1], L. YANG[1], D. MORROW[1], D. LI[1], J. D. KENNEDY[1], S. SWANSON[2], J. A. MONN[3];
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Abstract: Much evidence from preclinical work suggests that activation of metabotropic glutamate 2/3 (mGlu2/3) receptors could be an effective analgesic in chronic pain conditions. In this account, the analgesic properties of a novel mGlu2/3 receptor agonist prodrug were investigated. After oral absorption, the prodrug LY2969822 rapidly converts to the brain penetrant, potent, and subtype-selective mGlu2/3 agonist LY2934747 [1]. Behavioral assessments of allodynia, hyperalgesia, or nocifensive behaviors in preclinical pain models, after administration of prodrug LY2969822 as active equivalent doses of 0.3 - 10 mg/kg were conducted 1-4 hrs post treatment. In addition, the ability of i.v. administrated LY2934747 to modulate dorsal horn spinal cord wide dynamic range (WDR) neurons in naïve and spinal nerve ligated (SNL) rats was assessed for effects on spontaneous activity, electrically-evoked wind-up, and resulting after-discharge. Following a 1 or 10 mg/kg i.v. dose of LY2934747, the spontaneous activity and the electrically-evoked wind-up of WDR neurons in rats that had undergone spinal nerve ligation and developed mechanical allodynia was suppressed. Also, following a 10 mg/kg i.v. dose of LY2934747, the electrically-evoked wind-up of WDR neurons in naïve rats was significantly decreased compared to baseline measurements. In a model of sensitization, LY2969822 (p.o.) or LY2934747 (i.p.) prevented the nociceptive behaviors induced with intraplantar formalin injection with ED_{50} values of 3.2 mg/kg and 0.7 mg/kg, respectively. The on-target nature of this effect was confirmed by demonstrating pharmacological blockade with the mGlu2/3 antagonist LY341495. A 10 mg/kg dose of LY2969822 prevented tactile hypersensitivity with intraplantar capsaicin and reversed the SNL-induced tactile hypersensitivity. In addition, LY2969822, over a similar dose range (3-10 mg/kg), decreased mechanical hyperalgesia seen with intraplantar Complete Freund's Adjuvant (CFA) injection. The mGlu2/3 agonist prodrug demonstrated efficacy in visceral pain models, including a colorectal distension model and partially preventing the nocifensive behaviors in the mouse acetic acid writhing model. Partial or inconsistent decreases in nocifensive responses were seen in rat capsaicin-induced thermal hyperalgesia and the tactile hypersensitivity assessed 24 hours after incision in a rat post-surgical pain model. In conclusion, the mGlu2/3 agonist LY2934747 (delivered either i.p. or i.v.) and the prodrug LY2969822 (delivered p.o.) attenuated

pain behaviors across a broad range of pre-clinical pain models. [1] Monn JA et al, J. Med. Chem. 58:1776-94, 2015.

Disclosures: **M.P. Johnson:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **M.A. Muhlhauser:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **E.S. Nisenbaum:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **R.M.A. Simmons:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **B.M. Forster:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **K.L. Knopp:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **L. Yang:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **D. Morrow:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **D. Li:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **J.D. Kennedy:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **S. Swanson:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **J.A. Monn:** A. Employment/Salary (full or part-time):; Eli Lilly and Company.

Poster

705. Pain Models: Pharmacology

Location: Hall A

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Topic: D.08. Pain

Support: Grant-in-Aid (No. 26462726) from the Ministry of Education, Culture, Sport, Science, and Technology, Japan

Title: Possible involvement of spinal dopamine receptor subtypes in trigeminal nerve injury-induced mechanical hypersensitivity of rats

Authors: ***K. NAKAI**[1], A. NAKAE[2], T. KUBO[3], Y. MINEGISHI[1], Y. FUJINO[4], K. HOSOKAWA[3];
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Abstract: (Background) Peripheral branches of the trigeminal nerve may be damaged by maxillofacial injury or during surgical procedures and such damage may induce severe pain. Trigeminal neuropathic pain is very difficult to manage. Only antidepressants and anticonvulsants have been proved to be efficacious in treating trigeminal neuralgia. The dopamine receptor has been recognized to be important in the spinal modulation of nociceptive

transmission. Both pronociceptive and antinociceptive effects have been attributed to the dopamine receptor subtypes. The involvement of D1, D2, D3, and D4 receptors in trigeminal nociception and pain, particularly neuropathic pain, has been poorly investigated. Chronic constriction injury to the infraorbital nerve (ION-CCI) of rats induced mechanical hypersensitivity. It has proven a useful model for trigeminal neuropathic pain. The present study evaluated the possible role of spinal D1, D2, D3, and D4 receptors in ION-CCI rat model. (Material and methods) Male Sprague Dawley rats underwent unilateral CCI to the right ION. Two nylon (5-0) ligatures were tied around the ION. A series of von Frey filaments were used to determine pain hypersensitivity to mechanical stimulation on day 14 after surgery. The filaments were applied to skin innervated by the injured ION near the center of the vibrissal pad and surrounding the mystacial vibrissae. Head withdrawal, and touching or scratching of facial regions, were quantified as positive pain responses. A polyethylene (PE-10) catheter was implanted for upper cervical spinal injection of drugs. The rats were allowed to recover for 7 days. The time course of the antiallodynic effects and the dose-response effects of intrathecally administered a D1 receptor agonist SKF38393, a D1 receptor antagonist SCH23390, a D2 receptor agonist Sumanirole, a D2 receptor antagonist L-741626, a D3 receptor agonist 7-Hydroxy-DPAT, a D3 receptor antagonist PG01037, a D4 receptor agonist A 412997, and a D4 receptor antagonist L-745870 were examined. The time course data for the dose-response effects were analyzed by two-way analysis of variance and Tukey-Kramer multiple-comparison test. (Results and discussion) Intrathecal administration of SCH23390, L-741626, PG01037, and L-745870 increased mechanical thresholds in a dose dependent manner ($P < 0.05$). Intrathecal administration of SKF38393, Sumanirole, 7-Hydroxy-DPAT, and A 412997 did not alter mechanical thresholds. (Conclusion) The results indicated that spinal D1, D2, D3, and D4 receptors may play an important role in trigeminal nerve injury-induced mechanical hypersensitivity of rats.

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Poster

705. Pain Models: Pharmacology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 705.20/S1

Topic: D.08. Pain

Support: Uludag University- HDP-2013/17

Title: The contribution of Nucleus Raphe Magnus to the analgesia produced by centrally administered CDP-choline

Authors: *M. S. GURUN, M. K. GURBUZOGLU-ULKAN, D. BAGDAS, D. GOK-YURTSEVEN, O. EYIGOR;
Uludag Univ. Med. Fac., Bursa, Turkey

Abstract: Central cholinergic system and nicotinic acethylcholine receptor (nAChR) subtypes play significant roles in the process of pain control. These findings have been supported by pain studies in our laboratory in which we demonstrated the mediation of central nAChRs in analgesic and antihypereralgesic effects of CDP-choline. In addition, Nucleus Raphe Magnus (NRM), a region with dense cholinergic innervation which is localized to brainstem, has important functions including pain modulation, due to its strategic location and presence of connections. Therefore, the present study was designed to identify the possible role of NRM in analgesia elicited by intracerebroventricularly (icv) -administered CDP-choline and the underlying mechanism of its analgesic effect. For this purpose, we investigated whether or not cholinergic, opioidergic, Gabaergic and serotonergic antagonists microinjected into NRM alter the analgesic effects of CDP-choline (icv) in the acute pain model in rats. The effect of CDP-choline (icv) on acethylcholine and choline concentrations in NRM was analyzed by *in vivo* microdialysis study, as well. We found that the analgesic effect of intracerebroventricularly-administered CDP-choline in acute pain model was prevented by pretreatments with the nonspesific nicotinic receptor antagonist mecamilamine, the spesific α7 nicotinic receptor antagonist methyllicaconitin and the nonspesific serotonergic receptor antagonist methysergide microinjected into the NRM, while pretreatment with the nonspesific muscarinic receptor antagonist atropin microinjected into the NRM did not alter CDP-choline's effect. In addition, extracellular choline concentrations in NRM were incerased after intracerebroventricular CDP-choline admisinistration. In conclusion, our study shows for the first time the role of NRM and α7 nAChRs with serotonergic receptors located in the NRM in analgesic effect of intracerebroventricularly-administered CDP-choline in acute pain model. Supported by Research Foundation of Uludag University (HDP-2013/17)
keywords: CDP-choline, Nucleus Raphe Magnus, acute pain, analgesia

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Poster

706. Tactile Sensation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 706.01/S2

Topic: D.09. Tactile/Somatosensory Systems

Support: BIBS/NPNI New Frontiers Award

NASA RISG Consortium Fellowship

Title: A novel integrated EEG and neural modeling approach to uncovering the mechanisms of transcranial alternating current stimulation

Authors: *C. BLACK[1], U. AGRAWAL[1], M. LADOW[1], J. SANTOYO[1], J. VOIGTS[2], C. MOORE[1], S. JONES[1];
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Abstract: Transcranial alternating current stimulation (tACS), a non-invasive neuromodulation technique, has exhibited its utility as a therapeutic in a variety of applications: from enhancing vision in patients with optic neuropathy (Sabel 2011), to improving speech in patients with stroke (Fedorov 2010). While the benefits of tACS are evident, little is known of how these externally generated electric fields modulate on-going neural activity, making it difficult to select appropriate therapeutic stimulation parameters. Macroneurophysiological measures taken in conjunction with tACS will be crucial in this effort. Electroencephalography (EEG) provides a neural recording method with high-precision temporal resolution that can register the millisecond timescales inherent to neural activity, but concurrent EEG—tACS recording has many technical challenges, such as stimulation artifacts, amplifier irregularities, and long recording latencies, that impede effective incorporation of EEG. To elucidate the mechanism of action of tACS, we have designed and built a novel EEG – tACS technique to provide data for complementary biophysically realistic neural modeling. To create this technique, we developed a custom EEG system, based off the electrophysiology platform Open Ephys, with integrated real-time amplifier control and closed-loop capabilities. The amplifier control enables us to cycle our EEG amplifiers on and off within 200 microseconds. This means we can silence our recording amplifiers during tACS and then restart them in real-time to reliably capture the resulting neural data void of stimulation errors. Further, our closed-loop architecture provides a medium to drive parameterized stimulation from streaming neural data. This feedback allows us to probe the affect of tACS on EEG phase, amplitude, and frequency. We have implemented this technique to study the affects of tACS in primary somatosensory cortex (SI) on behavior during a non-painful tactile detection task. Preliminary results show modulation of tactile evoked responses and spectral dynamics in SI (see companion poster, J. Santoyo). To study the impact of tACS at cellular and network levels during tactile detection, we are employing a biophysically principled model of SI cortex that can emulate the recorded tactile evoked response and rhythms in SI (Jones et al, 2007, 2009, Ziegler et al 2010). Our goal is to link the observed changes in electrophysiology and behavior to the causal impact of tACS-induced electric fields on the underlying circuits. Ultimately, our combined methodologies will provide the framework to design rational stimulation protocols that optimize tACS-based therapies.

Disclosures: **C. Black:** None. **U. Agrawal:** None. **M. Ladow:** None. **J. Santoyo:** None. **J. Voigts:** None. **C. Moore:** None. **S. Jones:** None.

Poster

706. Tactile Sensation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 706.02/S3

Topic: D.09. Tactile/Somatosensory Systems

Support: 2013-2014 BIBS/NPNI New Frontiers Fund

Title: The role of muscular beta oscillations and modulation with transcranial alternating current stimulation in predicting tactile detection

Authors: *U. AGRAWAL[1], C. BLACK[2], M. LADOW[1], J. SANTOYO[3], C. KERR[4], C. MOORE[1], S. JONES[1];
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Abstract: Oscillations in the beta (15 - 29 Hz) band observed in primary somatosensory cortex as well as in the muscles have been shown to be functionally relevant for sensory perception and can be modulated with attention (Jones et al. 2010, van Ede et al. 2011, van Ede and Maris 2013). Recent attempts have successfully utilized transcranial alternating current stimulation (tACS) to impose these rhythms in cortex (Feurra et al. 2011) as well as modulate corticomuscular beta coherence, a measure of coordination between the cortex and the muscles (Wach et al. 2013). In this study, we investigated the role of muscular oscillations in perception and the causal impact of tACS induced cortical oscillations on the muscles. We applied tACS over somatosensory cortex during a tactile detection task while recording simultaneous electroencephalography (EEG) and electromyography (EMG). Our team designed and created a customized EEG system using Open Ephys (open-ephys.org), an open source initiative that is both flexible and inexpensive (companion poster, C. Black et al.). Using this custom built system, we were able to obtain simultaneous EEG, tACS, and EMG. We have previously shown that pre-stimulus beta activity in the cortex is predictive of successful perception of a tactile stimulus (Jones et al 2010). Here, we show preliminary data that suggests pre-stimulus beta oscillations in the muscles are also predictive of detected vs. non-detected tactile stimuli. Ongoing studies are aimed at investigating the causal impact of tACS in the muscles and on perception. Our findings provide preliminary evidence for a causal role of sensorimotor oscillations at the level of the muscles. Future studies may ultimately elucidate a clinical role for

EMG in diagnosis and therapeutics in disorders characterized by maladaptive beta rhythmicity, such as Parkinson's disorder.

Disclosures: **U. Agrawal:** None. **C. Black:** None. **M. Ladow:** None. **J. Santoyo:** None. **C. Kerr:** None. **C. Moore:** None. **S. Jones:** None.

Poster

706. Tactile Sensation

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Topic: D.09. Tactile/Somatosensory Systems

Support: 2013-2014 BIBS/NPNI New Frontiers Fund

Title: Testing the modulation of cortical dynamics and somatosensory perception using alpha and gamma-band transcranial alternating current stimulation

Authors: ***J. F. SANTOYO**[1], C. BLACK[2], U. AGRAWAL[3], M. LADOW[3], B. GREENBERG[4], S. JONES[3], C. MOORE[3];
[1]Cognitive, Linguistic & Psychological Sci., [2]Biomed. Engin., [3]Neurosci., [4]Psychiatry and Human Behavior, Brown Univ., Providence, RI

Abstract: Alpha and gamma band oscillatory activity in the sensory cortices are thought to play mechanistic roles in the gating of sensory information. Specifically, alpha-band activity (7-14Hz) has been associated with inattention to task-irrelevant sensory signals and gamma-band activity (30-80Hz) has been associated with enhancing the processing of task-relevant sensory signals (Siegel 2012). Here, we use transcranial alternating current stimulation (tACS), a non-invasive technique for inducing oscillatory activity to examine the role of these oscillatory dynamics in human sensory perception. Participants (n=10) performed a tactile detection task before and after tACS stimulation at either alpha or gamma-band frequencies. tACS was localized anatomically to S1 on the cortical hemisphere contralateral to the hand used for the tactile detection task. We analyzed changes in behavioral performance as well as changes in post-cue and pre-stimuli Event-Related Potentials (ERPs) and Event-Related Spectral Dynamics (ERSP) before and after tACS and between alpha and gamma-band stimulation conditions. Distinct elements of the post-cue and pre-stimulus ERPs and ERSPs were modulated by alpha and gamma-band stimulation in contralateral S1 but not ipsilateral S1. However, there was no observable influence of tACS stimulation on somatosensory perception. These preliminary results suggest that tACS can modulate functionally relevant oscillatory rhythms but potentially not behavior.

Disclosures: **J.F. Santoyo:** None. **C. Black:** None. **U. Agrawal:** None. **M. Ladow:** None. **B. Greenberg:** None. **S. Jones:** None. **C. Moore:** None.

Poster

706. Tactile Sensation

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Program#/Poster#: 706.04/S5

Topic: D.09. Tactile/Somatosensory Systems

Support: 2013 Faculty and Staff Research Award, Office of Research and Policy, College of Education & Human Development, University of Minnesota

Title: Robot-aided measurement of proprioceptive function in healthy and clinical populations

Authors: ***J. HOLST-WOLF**[1], I.-L. YEH[1], N. ELANGOVAN[1], J. AMAN[1], L. CAPPELLO[2,3], L. MASIA[3], J. KONCZAK[1];
[1]Kinesiology, Univ. of Minnesota, Minneapolis, MN; [2]Robotics, Brain and Cognitive Sci., Italian Inst. of Technol., Genova, Italy; [3]Robotics Ctr., Nanyang Technological Univ., Singapore, Singapore

Abstract: Diminished or absent proprioceptive information has severe consequences on many aspects of sensorimotor function leading to impairments in balance, locomotion and fine motor control. Despite the recognized importance of proprioception for intact motor behavior, the clinical assessment of proprioceptive deficits has been difficult. Recent advances in haptic robotic interfaces has enabled the use of such devices for the assessment of proprioceptive function. We recently demonstrated (Capello et al. 2015) that our patented wrist robotic system is capable of quantifying proprioceptive acuity of two degrees of freedom of the wrist. It yields thresholds that are valid (i.e., physiologically plausible), produces reliable data over repeated testing, and employs a protocol that generates objective data on proprioceptive status in less than 20 minutes (position or motion sense). Here we show that the system can be applied to a variety of clinical populations suspected of proprioceptive dysfunction by assessing the acuity of the wrist position sense. METHOD: From a neutral joint position, the wrist robot moved the wrist to two different joint positions (standard stimulus: 15 deg flexion, comparison stimuli > 15 deg) at a constant slow velocity of 6 deg/s. The participant then identified verbally which position had the larger amplitude. An adaptive algorithm generated the subsequent stimulus pair depending on the correctness of the previous responses. The assessment is typically complete after 30 trials. Based on the response and stimulus difference data a psychophysical just-noticeable difference threshold was determined. RESULTS: We here show exemplar threshold data of adult patients

with cortical stroke, Parkinson's disease, and peripheral sensory neuropathy and contrast their thresholds against a norm database of healthy adults (N = 22). We demonstrate that thresholds in these clinically populations can be quantified with precision in a timely manner. Expectedly, our data show that thresholds may be severely elevated with respect to a neurologically normal adult cohort. DISCUSSION: The purpose of this work is to demonstrate the clinical applicability of robot-aided proprioceptive assessment. From a clinical research perspective, this assessment system allows for the mapping of proprioceptive deficits in neurological disorders associated with proprioceptive deficits. From a clinical practitioner perspective, it allows for the objective assessment of proprioceptive function of individual patients. This implies that the system can be used as a diagnostic tool as well as a tool to monitor treatment efficacy.

Disclosures: J. Holst-Wolf: None. **I. Yeh:** None. **N. Elangovan:** None. **J. Aman:** 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.; Office of Research and Policy, College of Education & Human Development, University of Minnesota. **L. Cappello:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patent 62136065 SYSTEMS AND METHODS FOR ASSESSING AND TRAINING WRIST JOINT PROPRIOCEPTIVE FUNCTION – Serial No. 62/136,065. **L. Masia:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patent 62136065 SYSTEMS AND METHODS FOR ASSESSING AND TRAINING WRIST JOINT PROPRIOCEPTIVE FUNCTION – Serial No. 62/136,065. **J. Konczak:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patent 62136065 SYSTEMS AND METHODS FOR ASSESSING AND TRAINING WRIST JOINT PROPRIOCEPTIVE FUNCTION – Serial No. 62/136,065.

Poster

706. Tactile Sensation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 706.05/S6

Topic: D.09. Tactile/Somatosensory Systems

Support: 2013 Faculty and Staff Research Award from College of Education and Human Development University of Minnesota

Title: Robot-assisted assessment of proprioception: normative data in healthy adults

Authors: *I.-L. YEH[1], J. AMAN[1], N. ELANGOVAN[1], J. HOLST-WOLF[1], L. CAPPELLO[2], L. MASIA[3], J. KONCZAK[1];
[1]Univ. of Minnesota, Minneapolis, MN; [2]Italian Inst. of Technol., Genova, Italy; [3]Nanyang Technological Univ., Singapore, Singapore

Abstract: Proprioception is essential to intact motor function. While it is important to characterize proprioceptive acuity in healthy adults, no measurement is accepted universally as a gold standard. We have recently developed a reliable and valid assessment to measure wrist position sense with a wrist robot system (Capello et al. 2015). The purpose of this study is to establish a normative data set of proprioception in healthy adults using the wrist robot system. METHOD: The subject's dominant hand was moved passively by the robot inducing wrist flexion. After experiencing two passive wrist movements (standard stimulus: 15 deg flexion, comparison stimuli > 15 deg) by the wrist robot system, the subject identified which of two stimuli had the larger movement amplitude. An adaptive algorithm then generates the next stimuli differences depending on the correctness of response and magnitude of the stimulus difference. The JND threshold was determined as the estimated value on the last trial. The psychophysical just-noticeable-difference (JND) threshold for wrist joint position sense was the measure of proprioceptive acuity. RESULTS: 22 healthy adults (Age: 21- 62 y/o, average 44.6 ± 10.8 y/o, 7 Male/15 Female) were recruited. On average the position sense threshold for wrist flexion/extension: 1.719° ± 0.619°. The correlation between age and threshold was not significant for this sample population. DISCUSSION: A normative data set of proprioceptive acuity was established for young and middle-aged adults. This normative data set serves as a reference to determine the degree of the impairment in wrist position sense in clinical populations with suspected proprioceptive dysfunction.

Disclosures: I. Yeh: None. **J. Aman:** 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.; University of Minnesota, College of Education and Human Development, Office of Research & Policy. **N. Elangovan:** None. **J. Holst-Wolf:** None. **L. Cappello:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patent 62136065 SYSTEMS AND METHODS FOR ASSESSING AND TRAINING WRIST JOINT PROPRIOCEPTIVE FUNCTION – US Serial No. 62/136,065. **L. Masia:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ; US Patent 62136065 SYSTEMS AND METHODS FOR ASSESSING AND TRAINING WRIST JOINT PROPRIOCEPTIVE FUNCTION – US Serial No. 62/136,065. **J. Konczak:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patent 62136065 SYSTEMS AND METHODS FOR ASSESSING AND TRAINING WRIST JOINT PROPRIOCEPTIVE FUNCTION – US Serial No. 62/136,065.

Poster

706. Tactile Sensation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 706.06/S7

Topic: D.09. Tactile/Somatosensory Systems

Title: Potential applicability of laryngeal vibrotactile stimulation as a noninvasive therapeutic method for spasmodic dysphonia

Authors: *S. KHOSRAVANI, Y. TSENG, I. YEH, J. AMAN, J. KONCZAK;
Univ. of Minnesota, Minneapolis, MN

Abstract: Spasmodic dysphonia (SD) is a chronic neurological disorder accompanied with the involuntary spasm of vocal cords, depriving patients from producing normal speech-related items. Currently, temporary symptomatic treatment in form of botulinum toxin injection is the most prominent therapy for SD. While the origins of the disease are still in debate, SD is categorized as a type of focal dystonia (FD). The mentioned point directed our attention towards taking advantage of current knowledge about FD, in order to find potential treatment opportunities for spasmodic dysphonia. Huge body of research has revealed altered somatosensory processing in different forms of FD, including SD. This, together with the studies confirming the effectiveness of vibro-tactile stimulation (VTS) for the improvement of somatosensory perception in FD (and resultantly reduction of a number of dystonic symptoms), led us towards examining the effectiveness of VTS in SD. Here we first examined the unknown effect of VTS on cortical activity in healthy volunteers and then applied VTS to SD patients to measure the change in voice quality. Microvibrators were attached unilaterally to the skin above the thyroid. Brain signals were recorded using a 64-channel scalp EEG system over 4 seconds of vocalization followed by 4 seconds of vocalization plus vibro-tactile stimulation (total of 100 trials). Time-frequency analysis of the results revealed bilateral suppression of cortical activity in somatosensory and motor areas after vibration was applied. Suppressions were more pronounced in β and μ bands and on the contralateral side with respect to the side of stimulation. Second, three SD patients participated in an assessment protocol comprising three sets of 15-minute stimulation: VTS-no vocalization (5 min.) and vocalization with VTS (10 min.). Vocal quality was evaluated at the end of each trial in both ‘on’ and ‘off’ vibratory conditions. Patient self-reports on voice quality and recorded audio signals were analyzed a posteriori by a blinded rater using the Consensus Auditory-Perceptual Evaluation of Voice (Cape-V). Results showed improvement of self-reported voice quality for all participants and up to 35% improvement in

average Cape-V scores. Both tests provide preliminary evidence for the potential capability of using VTS as a non-invasive clinical treatment for spasmodic dysphonia.

Disclosures: S. Khosravani: None. **Y. Tseng:** None. **I. Yeh:** None. **J. Aman:** None. **J. Konczak:** None.

Poster

706. Tactile Sensation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 706.07/S8

Topic: D.09. Tactile/Somatosensory Systems

Title: Virtual reality visuomotor training improves proprioceptive acuity and transfers to untrained motor task

Authors: *N. ELANGOVAN[1], L. CAPPELLO[2], J. AMAN[1], L. MASIA[3], J. KONCZAK[1]; [1]Sch. of Kinesiology, Univ. of Minnesota, Minneapolis, MN; [2]Italian Inst. of Technol., Genova, Italy; [3]Nanyang Technological Univ., Singapore, Singapore

Abstract: BACKGROUND AND PURPOSE: When acquiring a new visuomotor skill, we face the challenge of aligning visual feedback with the movement-related proprioceptive feedback in order to generate motor commands that will eventually lead to precise movements that will achieve the movement goal. Here we address the question, if a training that relies on visual cues for learning will result in measurable changes in proprioceptive precision (acuity), and whether such improved proprioceptive function will translate into improved motor function. To address these questions, healthy adults engaged in a visuomotor training protocol requiring them to obtain precise wrist joint position. The purpose of the study was 1) to identify whether this training will improve proprioceptive acuity by quantifying training-related changes in limb position thresholds, and 2) to evaluate if proprioceptive changes are associated with motor performance of an untrained non-visuo motor task. METHOD: Using a wrist haptic robotic device, training involved tilting a virtual surface to position a virtual ball on a target by making small amplitude wrist flexion/extension movements. Tilt angle of the virtual surface reflected the participant's current wrist position. Proprioceptive acuity and precision of goal-directed active wrist movement in an untrained motor task were both assessed without vision before and after training. Specifically, wrist position sense discrimination thresholds were obtained using controlled passive robotic motion of the wrist. In addition, movement precision errors were determined as the difference between a 15° flexion target position and the final achieved joint position after an active movement to that same target. RESULTS: Improvements in

proprioceptive thresholds were found in all participants (N = 15; mean: pre/post = 2.1° ± 0.4°/ 1.4° ± 0.6°; a 33% improvement). Movement precision error improved in 82% of healthy participants (mean: pre/post = 3.1° ± 2.4° / 2.2° ± 2.4°; a 22% improvement). DISCUSSION: This study demonstrates: First, improvements in proprioceptive acuity are observable even after a brief sensorimotor training period. Second, enhanced motor performance in an untrained non-visuomotor task indicate that such improvements in proprioceptive function translate to improved movement precision. The results provide a scientific basis to apply such training to patients with known proprioceptive dysfunction.

Disclosures: **N. Elangovan:** None. **L. Cappello:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patent 62136065 SYSTEMS AND METHODS FOR ASSESSING AND TRAINING WRIST JOINT PROPRIOCEPTIVE FUNCTION – Serial No. 62/136,065. **J. Aman:** None. **L. Masia:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patent 62136065 SYSTEMS AND METHODS FOR ASSESSING AND TRAINING WRIST JOINT PROPRIOCEPTIVE FUNCTION – Serial No. 62/136,065. **J. Konczak:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent or license related to the work being reported is held by the authors L.C., L.M. and J.K. without direct corporate involvement at this time; US Patent 62136065 SYSTEMS AND METHODS FOR ASSESSING.

Poster

706. Tactile Sensation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 706.08/S9

Topic: D.09. Tactile/Somatosensory Systems

Support: NINDS NS090595

NINDS NS058668

Title: A push-pull pathway from somatosensory cortex to spinal trigeminal nuclei for motor control of the vibrissae

Authors: ***P. M. KNUTSEN**, N. MERCER LINDSAY, D. KLEINFELD;
Dept. of Physics, UCSD, La Jolla, CA

Abstract: Goal-directed behavior is an integral component of perception. Information transmitted by sensory afferents is shaped by external inputs as well as input from self-motion. In turn, sensory information guides motor output and thus the synthesis of behavior. Conceptually and anatomically, a sensorimotor system can be envisaged as a network of nested closed-loops that achieves postural stability during behavior. This control structure suggests the need for rapid computations of motor output based on the flow of sensory input. Vibrissa somatosensory cortex involves the convergence of exafferent, reafferent, and efference copy signals and is thus a candidate area for the computation of motor efferents. In particular, vibrissa somatosensory cortex (vS1) projects to and has been shown to influence both sensory input (Knutsen & Kleinfeld, SfN abstract 2013) and motor control (Matyas et al., Science 2010) via the brainstem spinal trigeminal complex (SpV). Here, we expand on prior observations of motor control via somatosensory cortex. Precise topographic maps of vibrissae representations in vS1 were obtained by imaging intrinsic optical signals through thinned-skull windows. These maps were used to guide through-skull, patterned optogenetic activation of L5b pyramidal cells in Thy1-ChR2 transgenic mice. We find that afferent and cortical activation of single units in the caudal aspect of spinal nucleus interpolaris (SpVIc) is topographically aligned. Further, units in the rostral aspect of SpVI (SpVIr) that were activated by stimulation of intervibrissal fur were also activated by optogenetic stimulation of the region of dysgranular cortex adjacent to the representation of E-row in vS1. Of particular interest, optogenetic activation of L5b cells in this region led to retraction of the vibrissae. Using a combination of anterograde indicators, we find that the activated cells project primarily into SpVIr and spinal oralis (SpVO), similar to projections seen from motor cortex (Mercer Lindsay et al., companion SfN abstract 2015). In contrast, the vibrissae responsive region of vS1 projects primarily into SpVIc (Sreenivasan et al., EJN 2015). In additional experiments, we find that activation of L5b cells in secondary somatosensory cortex (S2) led to protraction of the vibrissae. These cells project exclusively to spinal nucleus muralis (SpVM) that, like SpVIr, forms a direct connection with facial motoneurons (Pinganaud et al., JCN 1999; Matthews et al., JCN 2015). We conclude that the tandem combination of S1 and S2 enables bidirectional control of vibrissa movements via subregions of the spinal trigeminal complex, most likely via control of the set-point.

Disclosures: **P.M. Knutsen:** None. **N. Mercer Lindsay:** None. **D. Kleinfeld:** None.

Poster

706. Tactile Sensation

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Support: NINDS F31NS089316-01A1

CIHR MT-5877

NINDS NS090595

NINDS NS058668

NSF PolS 2014906

Title: Brainstem areas controlling exploratory nose motion in rodents

Authors: *A. KURNIKOVA[1], M. DESCHÊNES[2], J. D. MOORE[3], D. KLEINFELD[1]; [1]Neurosciences, UC San Diego, La Jolla, CA; [2]Univ. Laval, Québec, QC, Canada; [3]Harvard Univ., Boston, MA

Abstract: Rodents shift their nose from side to side when they actively explore. This motor action might be used to sample and lateralize odors efficiently in the space. Lateral motion of the nose appears to be correlated to breathing (Deschênes et al. Anatomical Record, 2014) and may contribute to odor sampling. The primary muscle responsible for nose deflection is the deflector nasi (Deschênes et al. Anatomical Record, 2014, Haidarliu et al. Anatomical Record, 2015). Motor neurons that drive deflection of the nose are located in the dorsal lateral division of the facial motor nucleus (Deschênes et al., manuscript in preparation). We study the premotor control of nose motion in order to further our understanding of synthesizing orofacial exploratory behaviors from individual motor actions. We identify putative premotor brainstem areas responsible for nose deflection through anatomical and functional characterization. Further study will examine how this circuitry is activated and modulated by the brainstem breathing centers and how the direction of nose motion is selected. Behaviorally, preliminary data show that air flow through the nares is modulated by lateral deflection of the nose. For anatomical tracing, we use replication competent rabies virus and pseudorabies virus to transsynaptically label secondary inputs to the deflector nasi muscle. We find secondary labeled cells in areas of the intermediate reticular formation (IRt), medial gigantocellular reticular formation (Gi), parvocellular reticular formation (PCRt) and rostral portion of the nucleus of the solitary tract (rNST). To determine the functional relevance of regions identified by anatomical tracing, we use AAV-ReaChR, a virally encoded red-shifted channelrhodopsin, to optogenetically activate cells in the candidate areas. We find that activating cells in a region that includes the IRt and Gi results in nose deflection towards the ipsilateral side in both awake and anesthetized mice. Ongoing studies will decipher the specific role of populations of neurons in these areas.

Disclosures: **A. Kurnikova:** None. **M. Deschênes:** None. **J.D. Moore:** None. **D. Kleinfeld:** None.

Poster

706. Tactile Sensation

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Program#/Poster#: 706.10/S11

Topic: D.09. Tactile/Somatosensory Systems

Support: NINDS NS090595

NINDS NS058668

Title: Projections from vibrissa motor cortex to the spinal trigeminal nuclei

Authors: *N. MERCER LINDSAY, P. M. KNUTSEN, D. GIBBS, H. J. KARTEN, D. KLEINFELD;
UCSD, La Jolla, CA

Abstract: Behavior is an outcome of efferent and afferent activation, sensory feedback, and predictive control. Our objective is to understand how behaviors are synthesized from individual motor actions. Here we focus on the circuits that drive one such action, whisking of the mystacial vibrissa. We demonstrate that the vibrissa motor cortex (vMC) projects to the spinal trigeminal nuclei, coincident with regions that are pre-motor to the facial motor nucleus (FMN) that controls the mystacial muscles. Using a combination of anterograde and retrograde viral indicators, we find that vMC projects to three subdivisions of the spinal trigeminal complex: the pars oralis (SpVO), interpolaris (SpVI), and muralis (SpVmu). In detail, a synaptophsyin-GFP virus injected into vMC showed labeled vMC synapses were most concentrated in the rostral interpolar nucleus (SpVIr). Optogenetic activation of the vMC to SpVIr pathway, via lentivirus pseudotyped with rabies glycoprotein that encoded a red-shifted channelrhodopsin (ReaChR), elicited both vibrissae protraction and retraction. We conclude that vibrissa motor cortex, along with vibrissa somatosensory cortex (Matyas et al. Science 2010; Knutsen, Mercer Lindsay, and Kleinfeld, companion SfN abstract, 2015), integrate with sensory inputs and modulate vibrissa motor output, most likely through control of the set-point, with subdivisions of the spinal trigeminal complex serving as premotor nuclei.

Disclosures: N. Mercer Lindsay: None. **P.M. Knutsen:** None. **D. Gibbs:** None. **H.J. Karten:** None. **D. Kleinfeld:** None.

Poster

706. Tactile Sensation

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Topic: D.09. Tactile/Somatosensory Systems

Support: Plastic Surgery Foundation

CIHR

Title: Designing an animal model to investigate corneal neurotization as a means of restoring sensation in patients with corneal anesthesia

Authors: *J. CATAPANO[1], M. P. WILLAND[2], A. ALI[2], T. GORDON[2], G. H. BORSCHEL[2]; [1]The Hosp. for Sick Children, North York, ON, Canada; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Patients with corneal anesthesia are susceptible to corneal injury, causing corneal scarring with progressive vision loss. Standard therapy, including corneal transplantation, fails to prevent vision loss in these patients. Dr. Borschel and Dr. Ali have developed a novel surgical procedure to restore sensation to the cornea using nerve grafts and transfers from the contralateral sensate face. All patients have demonstrated improvements in corneal sensation and vision has stabilized or improved in all patients. An animal model of corneal neurotization is needed in order to investigate how nerve regrowth impacts corneal epithelial maintenance and healing. Here, we describe our initial work establishing a model of corneal denervation in the rat. Removing the corneal innervation is a necessary first step prior to reconstructing the corneal innervation with neurotization. Thy1-GFP+ rats, which express green fluorescent protein (GFP) in neurons and axons, were used to investigate a method of corneal denervation by transecting the ciliary branches of the trigeminal nerve, which innervate the cornea. Alcohol (90% and 100% EtOH) was applied to the transected ciliary nerves in a subset of animals to further damage the ciliary nerves following injury. The denervated and uninjured cornea were harvested at 1, 2, 3 and 4 weeks to assess the cornea for axon regeneration. Ciliary nerve transection resulted in complete loss of GFP+ axons in the cornea 1 week after injury and axon regrowth into the cornea was apparent at 2 weeks. Alcohol exposure to the ciliary nerves following transection delayed axon regrowth into the cornea, however at 4 weeks axon regrowth was also evident in those animals treated with alcohol. Ciliary nerve transection may be used as a model of spontaneous corneal nerve regeneration, however to investigate corneal neurotization in a model that recapitulates the clinical scenario, a method of corneal denervation that does not permit spontaneous corneal reinnervation must be developed.

Disclosures: **J. Catapano:** None. **M.P. Willand:** None. **A. Ali:** None. **T. Gordon:** None. **G.H. Borschel:** None.

Poster

706. Tactile Sensation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 706.12/S13

Topic: D.09. Tactile/Somatosensory Systems

Support: NSF Grant IOS 1257886

NSF Grant IOS 1425049

NSF IGERT Grant DGE 0903637

Title: Proprioceptive tuning to appendage mechanics: a comparison of pectoral fin proprioception in fishes

Authors: *B. R. AIELLO, M. W. WESTNEAT, M. E. HALE;
Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Proprioceptive feedback is critical to the motor performance of many animals. While proprioception has been documented in the pectoral fins (forelimbs) of fishes, pectoral fin morphology, mechanics, and kinematics vary markedly among species and it is unclear how such variation is associated with sensory input. In other sensory systems, input has been shown to be tuned to the most likely range of stimuli that define the organism's behavioral repertoire. Here we test the hypothesis that neuromechanical tuning occurs in fin-based proprioception. To test this hypothesis, we compare the proprioceptive afferent response to fin ray bending in a pair of closely related wrasses that employ dichotomic swimming behavior, the rowing *Halichoeres bivittatus* and the flapping *Gomphosus varius*. In both species, phasic afferent activity was observed in response to sinusoidal stimuli between 3 and 6Hz, which corresponds to the range of fin beat frequencies observed in these species. This reveals that proprioceptive feedback can be encoded at biologically relevant fin bending frequencies that mimic natural swimming behavior. In response to step-and-hold stimuli, a burst of spikes occurred in both species when the fin was raised from and again when it was returned to its resting position. The duration and number of spikes of these bursts as well as the spike rate over the hold period of the stimulus (3.5s) increased with increasing bending amplitude. Spike sorting revealed the presence of both slowly and rapidly adapting afferent activity during the step-and-hold stimuli. An interspecific comparison of fin mechanics revealed that pectoral fin ray flexural stiffness of the flapper, *G. varius*, was an order of magnitude stiffer in comparison to the rower, *H. bivittatus*, which relates to the greater fin bending amplitude observed in *G. varius* during swimming. A comparison of the step-and-hold stimuli between species revealed that it required a four times larger bending

amplitude to elicit afferent activity in *H. bivittatus* than in *G. varius*. A larger bending amplitude was also required to elicit sustained activity during the hold period in *H. bivittatus*. The results of this study suggest that sensory physiology can be tuned to the fin mechanics through adapting sensitivity of the proprioceptive system.

Disclosures: B.R. Aiello: None. **M.W. Westneat:** None. **M.E. Hale:** None.

Poster

706. Tactile Sensation

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Topic: D.09. Tactile/Somatosensory Systems

Support: IOS 1257886

NSF IGERT DGE-0903637

Title: Tactile sensation in a flexible biological membrane: Investigating touch in fish pectoral fins

Authors: *A. R. HARDY, B. M. STEINWORTH, M. E. HALE;
Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Research on limb tactile sensation has focused on mammalian digits, yet studies of other limb structures may provide alternative prospective on how tactile sensors can be organized and function. In some fishes, pectoral fins (paired forelimbs) function as locomotor structures to provide thrust and/or maneuverability. It has been shown that the activity of sensory nerves innervating these propulsive fins reflects the amplitude and velocity of fin ray bending during locomotion, providing proprioceptive feedback on fin movement. In other taxa, fins do not function as propulsive locomotor structures but remain morphologically robust. We hypothesized that, in these cases, fins function primarily as flexible sensory membranes that provide tactile information about contacted objects and fluid movement. To test this novel idea about fin sensory function, we investigated the pectoral fins of the pictus catfish (*Pimelodus pictus*), a benthic species that lives in close association with the substrate. Behavioral, neuroanatomical, and electrophysiological data indicate the pectoral fin of *P. pictus* functions as a membranous sensory surface. During forward swimming, *P. pictus* holds its pectoral fins laterally at 33° ± 11.5° relative to the body axis. This relatively constant angle suggests that the pectoral fins are not primarily motor structures. Immunostaining revealed nerves and putative

mechanoreceptors present throughout the pectoral fin. Nerves run parallel to the fin rays and often terminate on structures that positively stain with an antibody to cytokeratin 20, a Merkel cell marker. In an effort to elucidate the role of this innervation and sensory morphology we performed multi-unit electrophysiological recordings from nerves innervating the pectoral fin. Fish were exposed to step-and-hold and ramp-and-hold stimuli to examine fin ray responses to shear along the fin ray surface, static pressure, and fin ray bending. Stimuli were chosen based on behavioral observations to mimic the hypothesized forces the pectoral fin might encounter during swimming. Nerves respond with an increase in burst duration as fin ray bending amplitudes increase. In contrast to fishes that utilize their fins primarily for locomotion, pictus afferents also exhibit a vigorous response to both shear and static pressure. This ability to perceive tactile stimulation suggests that fish receive ample mechanosensory feedback about their environment as the pectoral fin moves over the substrate. Their organization and physiology may inform the design of engineered sensory surfaces, particularly for use in aquatic environments.

Disclosures: **A.R. Hardy:** None. **B.M. Steinworth:** None. **M.E. Hale:** None.

Poster

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Support: NSF CRCNS-IIS-1208118

NSF CAREER-IOS-0846088

Title: Mechanical response of isolated rat whiskers to airflow: bending direction, bending magnitude and vibration frequency

Authors: ***Y. S. W. YU**[1], M. M. GRAFF[1], M. J. Z. HARTMANN[1,2];
[1]Dept. of Mechanical Engineering, Northwestern Univ., [2]Dept. of Biomed. Engin., Northwestern Univ., Evanston, IL

Abstract: Whiskers have been the subject of research in both rodents and pinnipeds, but studies in these two clades have generally focused on separate aspects of whisker function. Studies in rodents have focused primarily on the use of whiskers as contact sensors and have investigated questions in active tactile perception and sensorimotor integration. Studies in pinnipeds have

focused primarily on the use of whiskers as remote flow sensors. To date, few if any studies have investigated the possibility that rodents might also use their vibrissae as flow sensors. If air currents of the magnitude typically found in natural environments generate significant motion of the vibrissae, then an important component of rodent vibrissal sensation may have been overlooked. The present work takes the first steps towards investigating the possibility that vibrissae may play a role in airflow sensing in rats. We quantified the mechanical responses of five isolated rat whiskers obtained from a female Long-Evans rat. Whiskers were placed at different orientations relative to the airflow, and airflow speed was varied between 0.4 and 5.7 m/sec. Results indicated that the whisker bends in the direction of the airflow. The magnitude of the bending depends on airspeed, the orientation of the whisker relative to the airflow, and geometric parameters of the whisker. In the presence of airflow, the whisker also vibrates around its new static ("bent") position, with vibrations occurring near the whisker's resonance modes. We combined these results with an anatomical model of the rat head and whisker array to simulate how whiskers' mechanical responses to airflow will vary across the entire vibrissal array. Results are discussed in the context of a potential functional role for vibrissae in anemotaxic behavior.

Disclosures: Y.S.W. Yu: None. **M.M. Graff:** None. **M.J.Z. Hartmann:** None.

Poster

706. Tactile Sensation

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NSF EFRI-0938007

Title: Responses of trigeminal ganglion neurons to the 3D mechanics of whiskers

Authors: *N. E. BUSH[1], L. A. HUET[2], A. E. T. YANG[2], P. KUMARAPPAN[3], J. A. ELLIS[1], M. J. Z. HARTMANN[2,3];

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Abstract: During tactile exploration, rats and mice rhythmically brush and tap their whiskers (vibrissae) against objects in a behavior known as whisking. Although whisking motions are primarily rostrocaudal, contact with an object will often deflect the whisker significantly out of its plane of rotation. This deflection will cause significant forces and torques in all three dimensions (3D). These mechanical signals will be transmitted to the primary sensory neurons that respond to whisker deflection, which reside in the trigeminal ganglion (Vg). At the same time, it is well known that Vg neurons exhibit strong angular tuning. In other words, they respond more strongly to whisker deflections in one direction than the others. It is therefore clear that an understanding of coding properties of Vg neurons hinges on characterizing their response to whisker deformations in 3D. To this end, our laboratory has developed a mechanical model of whisker bending that allows us to compute all three components of force and all three components of torque at the whisker base given the whisker's 3D shape. The present work takes the first steps towards characterizing how Vg neurons encode both large and small angle deflections in response to forces and torques in 3D. We record from Vg cells in the anesthetized rat while manually deflecting the whisker in multiple directions and while varying deflection amplitude and velocity. To quantify the mechanical stimuli applied to the whisker, we record high-speed video of the whisker in multiple high-speed cameras. We track the whisker in these separate videos and create a 3D reconstruction of whisker shape using stereo videography techniques. Finally, we apply our 3D mechanical model to the extracted shape of the whisker to compute the forces and the torques at the base of the whisker. We describe initial efforts to correlate these mechanical and geometrical signals with the responses of Vg neurons.

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Poster

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NSF CAREER IOS-0846088

Title: Evidence for tuning to stimulus directionality in the responses of neurons with multi-whisker receptive fields in spinal trigeminal nucleus interpolaris

Authors: *C. S. BRESEE[1], N. E. BUSH[1], A. S. KALOTI[1], E. C. JOHNSON[4], S. N. NAUFEL[2], M. G. PERICH[2], D. L. JONES[4], M. J. Z. HARTMANN[2,3];
[1]Interdepartmental Neurosci. Program, [2]Biomed. Engin., [3]Mechanical Engin., Northwestern Univ., Evanston, IL; [4]Dept. of Electrical and Computer Engin., Univ. of Illinois at Urbana Champagne, Urbana, IL

Abstract: The rat vibrissal-trigeminal system is an important model for the study of somatosensation, yet to date we have little understanding of how tactile information from multiple whiskers is integrated at various stages of the system. It is well-established that primary sensory neurons in the trigeminal ganglion respond to one and only one whisker. It is also well known that trigeminal ganglion neurons exhibit strong angular tuning, meaning that responses are much stronger when the whisker is displaced in one direction compared to others. Trigeminal ganglion neurons send signals to the trigeminal brainstem nuclei, the first structures that exhibit multi-whisker receptive fields. In the present work, we aimed to begin to quantify responses of neurons in spinal trigeminal nucleus interpolaris (SpVi) of awake rats under a wide variety of behavioral conditions. We were particularly interested in assessing how the strong angular tuning seen in ganglion responses might be reflected in the multi-whisker responses of SpVi neurons. We recorded from SpVi during behaviors that included non-contact whisking, nose-poking, whisking against a flat vertical surface (a wall), and grooming. We simultaneously recorded electromyographic (EMG) activity from both intrinsic and extrinsic musculature. Results showed that very little if any spiking occurred during non-contact whisking, despite robust and rhythmic EMG activity. Activity levels increased during multi-whisker contact with objects, including nose poking and contact with the wall. Activity appeared to be particularly enhanced during grooming behavior, in which the paws typically stimulate many whiskers across the array in a directional sequence. To more carefully quantify the extent to which multi-whisker receptive field neurons in the trigeminal nuclei reflected the direction of stimulation across the array, we performed experiments in the anesthetized animal. A vertical rod was swept through the full vibrissal array at varying speeds in the rostral-caudal and caudal-rostral directions. Recordings from 15 neurons in the brainstem trigeminal complex showed that majority of cells (80%) were tuned for stimulus direction, typically responding more strongly when stimulated from caudal to rostral. Together, results from the awake and anesthetized animal provide some preliminary support for the hypothesis that SpVi neurons integrate information about the angular tuning of single whiskers into a code for stimulus directionality across larger groups of neighboring whiskers.

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Poster

706. Tactile Sensation

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Topic: D.09. Tactile/Somatosensory Systems

Support: NSF Grant IOS-1150209

Title: Inferring the neural representations underlying perceptual invariance in touch

Authors: ***H. P. SAAL**, J. D. LIEBER, Z. M. BOUNDY-SINGER, A. I. WEBER, S. J. BENSMAIA;
Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: A crucial aspect of perception is the recognition of invariant features based on an ever-changing stream of sensory input. For example, we can tell an object's identity from different viewpoints, or recognize a friend's voice independent of the exact words spoken. In the sense of touch, the perceived roughness of a surface is remarkably independent of the movements that are used to probe the surface. While roughness ratings have been shown to be invariant to scanning speed, the peripheral neural representation of texture is not: changes in speed affect both the firing rates and the timing of afferent responses, raising the question of how the well-documented perceptual invariance arises. One possibility is that information about scanning speed, which is also conveyed in peripheral responses, is extracted by the brain and then used to interpret the texture signal. However, our perception of scanning speed turns out to be biased and imprecise, especially for finer textures, so it is unclear whether it can support this hypothetical process. Here, we take a different approach and ask whether we can arrive at speed-independent texture representations without the need for a precise estimate of scanning speed. To this end, we record the responses of cutaneous mechanoreceptive afferents - namely SA1 (slowly-adapting type 1), RA (rapidly-adapting), and PC (Pacinian) fibers - to a diverse set of textured surfaces delivered to the fingertip skin at three different scanning speeds. We also present subsets of these textures at different speeds to human subjects and ask them to rate either the roughness of the texture or the speed at which it is scanned across the skin. We consider the peripheral input as consisting of three signals, namely the SA1, RA, and PC population responses, and then try to separate speed and texture information using different linear combinations of these signals. We find that it is possible to derive features that are almost

completely speed-invariant. Furthermore, speed-invariant features mirror perceptual ratings of roughness and speed-dependent features mirror perceptual ratings of scanning speed. Our results explain how roughness ratings can be invariant with respect to scanning speed without a precise estimate of scanning speed. Furthermore, we offer a new interpretation of roughness as a perceptual signal that arises from simultaneously optimizing the information about texture and its invariance with respect to exploratory parameters.

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Poster

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Topic: D.09. Tactile/Somatosensory Systems

Support: NINDS RO1 NS 082865

Title: Neurons in primary somatosensory cortex encode complex hand postures and movements

Authors: ***G. TABOT**[1], J. M. GOODMAN[2], A. S. RAJAN[2], A. K. SURESH[2], N. G. HATSOPOULOS[1], S. J. BENSMAIA[1];
[1]Organismal Biol. and Anat., [2]Committee on Computat. Neurosci., Univ. of Chicago, Chicago, IL

Abstract: The ability to dexterously manipulate objects relies heavily on somatosensory signals from the hand. Without these signals, activities of daily living like turning a doorknob or brushing one's teeth would be slow, clumsy, and effortful. A unique aspect of somatosensory system is that it comprises a deformable sensory sheet: cutaneous signals must be integrated with proprioceptive ones to achieve a three-dimensional representation of objects grasped in the hand. To begin to understand how this integration happens, we must first understand how hand postures and movements are encoded in primary somatosensory cortex (S1). To this end, we record the responses of S1 neurons and track hand movements while monkeys grasp objects varying widely in shape and size, thereby eliciting different hand movements. We use a variety of analytical methods to determine what aspects of the kinematics drive individual proprioceptive neurons. We find that proprioceptive neurons respond to movements of multiple joints of the hand. Furthermore, proprioceptive neurons are driven not only by hand postures (angles of several joints) but also by movements (the derivatives of joint angles). Our results are

consistent with the idea that S1 neurons encode complex hand postures distributed over multiple digits with the ultimate goal of encoding the positions of the fingertips relative to one another.

Disclosures: G. Tabot: None. **J.M. Goodman:** None. **A.S. Rajan:** None. **A.K. Suresh:** None. **N.G. Hatsopoulos:** None. **S.J. Bensmaia:** None.

Poster

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Topic: D.09. Tactile/Somatosensory Systems

Support: NSF Grant IOS-1150209

Title: The coding of natural textures in primate somatosensory cortex

Authors: *J. LIEBER, H. P. SAAL, S. J. BENSMAIA;
Univ. of Chicago, Chicago, IL

Abstract: A major question in sensory neuroscience is how we extract perceptually consistent experiences from continuously changing sensory input. This perceptual invariance is well-illustrated by the experience of tactile texture. Indeed, the response of cutaneous mechanoreceptors is highly dependent on the speed and force with which a texture is explored. In contrast, the perception of texture is almost completely independent of these exploratory parameters. Thus, somewhere along the neuraxis, texture information must be disentangled from these exploratory parameters to produce an invariant perceptual representation. To investigate the neural mechanisms underlying texture invariance, we scanned a large set of natural and artificial surfaces across the fingertip of Rhesus macaques while recording the responses evoked in single-units in primary somatosensory cortex (areas 3b, 1 and 2). Then, using machine learning approaches, we found that textures can be classified based on the responses they evoke in small populations of S1 neurons, showing that these neurons carry rich information about texture in both the strength and temporal patterning of their responses. Moreover, we found that, while the texture-specific temporal spiking patterns change with scanning speed, the mean rate elicited by textures is constant across different speeds. The speed-independence of firing rates in S1 stands in contrast to the strong speed dependence of peripheral responses. It suggests that the cortical representation of texture is more invariant to speed than is its peripheral counterpart. Finally, we evaluated the extent to which the texture representation in S1 could account for perceptual judgments of texture obtained from human subjects. We found that perceived

roughness is better predicted from the responses of neural populations in area 1 than in area 3b, further bolstering the hypothesis that there is a progressive elaboration of texture information as one ascends the somatosensory neuraxis.

Disclosures: J. Lieber: None. **H.P. Saal:** None. **S.J. Bensmaia:** None.

Poster

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Topic: D.09. Tactile/Somatosensory Systems

Support: Kimberly Clark Corporation

Title: A model that simulates the response of the somatosensory nerves to arbitrary spatio-temporal deformations of the skin of the hand

Authors: *B. P. DELHAYE, H. P. SAAL, B. C. RAYHAUN, S. J. BENSMAIA;
Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Tactile information is conveyed by different classes of primary afferents in the somatosensory nerves. Afferent responses are driven by two processes: (1) stimulation applied to the skin's surface causes it to deform, producing time-varying stresses and strains at the location of mechanoreceptors, and (2) these stresses and strains are transduced by the receptors to produce highly precise and repeatable spike trains in afferents. Here, we built a model that simulates the response of afferents sampled over the entire hand to any arbitrary spatio-temporal deformations of the skin. To this end, the model replicates the two stages of mechanotransduction. First, the stresses resulting from the stimulus at the receptor location are estimated as the sum of two components: a quasi-static component caused by the redistribution of pressure applied at the skin's surface and a dynamic component resulting from the variation of pressure with time, taking into account the biomechanical properties of the skin and *in vivo* quantification of how vibrations propagate across the skin. Second, the resulting stresses are used as input to integrate-and-fire models that produce as output the spiking responses of individual afferents. We show that the model accounts for the response of all three classes of afferents (SA1, RA and PC) to both static and dynamic stimuli. We demonstrate that simulated responses replicate measured responses to complex spatio-temporal stimuli, such as scanned braille or natural textures. The model will be invaluable for providing biomimetic somatosensory feedback in hand neuroprostheses by converting the output of sensors into biomimetic responses that can

then be effected in the nerve through electrical stimulation. The model will also be an important tool in somatosensory research by providing a fast and accurate simulation of the response evoked in the entire population of cutaneous mechanoreceptive afferents by any tactile stimulus.

Disclosures: B.P. Delhaye: None. **H.P. Saal:** None. **B.C. Rayhaun:** None. **S.J. Bensmaia:** None.

Poster

706. Tactile Sensation

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Topic: D.18. Brain-Machine Interface

Support: DARPA

Title: What can bionic fingers tell us about objects? Extracting behaviorally relevant features from finger sensors output

Authors: B. P. DELHAYE[1], *E. W. SCHLUTER[2], M. S. JOHANNES[3], K. D. KATYAL[3], F. V. TENORE[3], S. J. BENSMAIA[1];
[1]Organismal Biol. and Anat., [2]Computer Sci., The Univ. of Chicago, Chicago, IL; [3]Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Abstract: Efforts are underway to restore sensorimotor function in amputees and tetraplegic patients using anthropomorphic robotic hands. For this approach to be clinically viable, however, not only must control signals be sent to the hands (through a neural or muscle interface), but sensory signals from the hand must also be conveyed back to the patient. To convey tactile feedback necessary for object manipulation, behaviorally relevant information must be extracted in real time from the output of sensors on the prosthesis. In the present study, we recorded the sensor output from two different bionic fingers (one by SynTouch, the other by APL) during the presentation of a variety of different tactile stimuli, including indentations, vibrations, textures, and moving gratings. Furthermore, the parameters of stimulus delivery (location, speed, direction, indentation depth, vibration frequency and amplitude, etc.) were systematically varied. We then developed simple algorithms to extract behaviorally relevant features from these outputs, such as the stimulus location, surface texture, motion speed, and motion direction. We then assessed the degree to which these algorithms could reliably extract these different types of sensory information across different conditions of stimulus delivery. Not only do our results have

important implications for the development of hand neuroprostheses, but they also shed light on the problems the brain has to solve to extract information from natural afferent responses.

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Poster

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Program#/Poster#: 706.22/T3

Topic: D.09. Tactile/Somatosensory Systems

Support: NIH R01 NS 082865

Title: Do proprioceptive neurons in somatosensory cortex encode muscle length?

Authors: ***J. GOODMAN, JR**, G. A. TABOT, A. S. RAJAN, A. K. SURESH, N. G. HATSOPOULOS, S. J. BENSMAIA;
Univ. of Chicago, Chicago, IL

Abstract: Somatosensory signals from the hand are critical to our ability to dexterously manipulate objects. Proprioceptive signals from the hand convey information about hand posture and movements that are integrated with cutaneous signals to culminate in a three-dimensional representation of objects grasped in the hand. While tactile response properties in primary somatosensory cortex (S1) have been extensively characterized, their proprioceptive counterparts have not, particularly for the hand. To fill this gap, we record the responses of S1 neurons (areas 3a and 2) and track hand movements while monkeys grasp objects varying widely in shape and size, thereby eliciting different hand movements. Using a musculoskeletal model, we reconstruct the lengths of hand muscles and tendons during the grasp. We then assess the degree to which musculotendon lengths are related to S1 responses in two ways: First, we assess the degree to which neuronal responses can be predicted based on musculotendon lengths. Second, we assess the degree to which musculotendon lengths can be decoded from the activity of populations of S1 neurons. Finally, we compare the performance of the musculotendon-based models to models of S1 responses based on kinematics.

Disclosures: **J. Goodman:** None. **G.A. Tabot:** None. **A.S. Rajan:** None. **A.K. Suresh:** None. **N.G. Hatsopoulos:** None. **S.J. Bensmaia:** None.

Poster

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Topic: D.09. Tactile/Somatosensory Systems

Title: Tactile responses of single neurons in the ventral caudal nucleus of awake humans

Authors: J. S. NEIMAT[1], J. P. NOEL[2], H. P. SAAL[3], J. F. DAMMANN, III[3], S. J. BENSMAIA[3], *M. A. HARVEY[1];
[1]Neurolog. Surgery, [2]Vanderbilt Brain Inst., Vanderbilt Univ. Med. Ctr., Nashville, TN;
[3]Organismal Biol. and Anat., Univesity of Chicago, Chicago, IL

Abstract: Information about texture is acquired by the active scanning of the fingers across an object's surface. These scanning movements elicit high frequency, complex vibrations in the skin which are converted into neural signals by cutaneous mechanoreceptors. In non-human primates the frequency content of these skin vibrations are represented in the precise timing of the elicited action potentials both at the periphery, as well as at the earliest stages of cortical processing. While peripheral and cortical neurons exhibit similar responses to vibrations, there are also important differences. For example, the firing rates of peripheral afferents are modulated by both vibratory amplitude and frequency, while firing rates in cortex depend only on amplitude, independently of frequency. Furthermore, individual cortical neurons respond to a much wider range of frequencies than any one class of afferents, suggesting convergence of submodality input onto individual neurons in cortex. To better understand how information is transformed between the periphery and cortex, we deliver vibrations, ranging from simple sinusoids to band-pass noise stimuli and varying in intensity and frequency composition, while recording the responses evoked in individual neurons of the ventral caudal nucleus (Vc, equivalent to VPL/VPM) in awake human patients undergoing deep brain stimulation surgery for essential tremor. We then characterize the strength and temporal patterning of thalamic responses to the wide range of stimuli and compare these to their peripheral and cortical counterparts. We show that the thalamus is not a simple relay for sensory information but rather contains an elaborated representation of tactile stimuli.

Disclosures: J.S. Neimat: None. **J.P. Noel:** None. **H.P. Saal:** None. **J.F. Dammann:** None. **S.J. Bensmaia:** None. **M.A. Harvey:** None.

Poster

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Support: Chicago Biomedical Consortium Grant - 049

Pritzker Fellowship

Title: Tactile coding in the cuneate nucleus of macaques

Authors: *A. K. SURESH[1], T. TOMLINSON[2], J. WINBERRY[1], J. M. ROSENOW[2], L. E. MILLER[2], S. J. BENSMAIA[1];
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Abstract: A central question in neuroscience is how sensory representations are transformed as they ascend the neuraxis. In primates, the coding of tactile information has been extensively studied in primary afferents and in primary somatosensory cortex (S1). The general picture that emerges from this previous work is that the responses of neurons in S1 explicitly encode certain behaviorally relevant stimulus features - for example edge orientation or direction of movement - that are either not encoded in the responses of individual afferents, or are encoded in a much more primitive way. A fundamental question, then, is to what extent this process of elaboration of sensory representations occurs at intermediate processing stages. Very little is known about the response properties of neurons in the two nuclei that are interposed between the periphery and cortex, namely, the cuneate nucleus (CN) and the ventroposterior lateral nucleus of the thalamus. In the present study, we seek to fill this gap by investigating the response properties of neurons in the cuneate nucleus (CN) of awake behaving monkeys. We seek to understand (1) whether single neurons in CN receive convergent input from different cutaneous submodalities, and (2) whether CN responses are selective for the higher order stimulus features encoded by cortical neurons. To this end, we have developed an approach to implant electrode arrays chronically in the CN and recorded the responses of CN neurons to a wide variety of stimuli that have previously been used to investigate tactile coding at the periphery and in cortex, including skin vibrations, oriented edges, moving gratings, and textures. We find that that individual CN neurons exhibit properties that are indicative of convergent input from multiple cutaneous submodalities and exhibit tuning for behaviorally relevant features, properties that are not observed at the periphery. Our results demonstrate that the CN is not simply a relay station for somatosensory information but rather is instrumental in its processing and elaboration.

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Poster

707. Basal Ganglia: Input Integration

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Title: Depression of corticostriatal transmission and responsiveness of striatal projection neurons to phosphodiesterase 10A inhibition in neuronal nitric oxide synthase knockout mice

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Abstract: The striatum contains a rich variety of cyclic nucleotide phosphodiesterases (PDEs) which play a critical role in the regulation of cAMP and cGMP signaling. PDEs modulate striatal medium-sized spiny neuron (MSN) excitability, as well as glutamatergic corticostriatal transmission by limiting the diffusion of cAMP/cGMP within subcellular domains. We previously demonstrated that pharmacological inhibition of PDE10A robustly increases the responsiveness of MSNs recorded *in vivo* to excitatory corticostriatal transmission driven by stimulation of the frontal cortex. However, the signaling mechanisms underlying PDE10A inhibitor-induced changes in corticostriatal transmission are only partially understood. The current studies assessed the role of the nitric oxide (NO)-cGMP signaling pathway in the above effects using wild type (WT) and neuronal NO synthase (nNOS) knock-out (KO) mice. WT and KO mice were treated with vehicle or the potent and selective PDE10A inhibitor TP-10 (3.2 mg/kg, s.c.). Biochemical studies showed that basal and TP-10-evoked tissue cAMP levels were similar in WT and nNOS KO mice. Conversely, both basal and TP-10-evoked cGMP levels were robustly decreased in nNOS KO mice as compared to WT controls. In electrophysiological studies, WT and nNOS KO mice were anesthetized with urethane and single-unit spike activity was isolated during low frequency electrical stimulation of the ipsilateral motor cortex.

Recordings of MSNs performed in nNOS KO mice revealed that both population firing activity and cortically-evoked responses were depressed as compared to WT controls, indicating that in the intact striatum NO signaling plays a key role in mediating synaptic facilitation during stimulation of corticostriatal pathways. We next tested if upstream NO derived from nNOS is necessary for the facilitatory effects of PDE10A inhibition on cortically evoked firing. Comparisons of the effects of TP-10 administration across genotypes indicated that MSNs recorded in nNOS KO mice were considerably less responsive to drug than WT controls, indicating that NO-derived from nNOS is necessary for the facilitatory effects of PDE10A inhibition on cortically evoked firing. Taken together, these observations provide further evidence that tonic NO signaling acts to facilitate corticostriatal transmission in the intact striatum via a cGMP-dependent mechanism. Furthermore, PDE10A acts to filter asynchronous or weak cortical input to MSNs by specifically suppressing this facilitatory influence of NO-sGC-cGMP signaling.

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Poster

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IMPULSA03

Title: Intrinsic properties of striatal neurons determine similar corticostriatal and thalamostriatal postsynaptic suprathreshold responses

Authors: *M. A. ARIAS, J. BARGAS, D. TAPIA, E. GALARRAGA;
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Abstract: The striatum integrates cortical and thalamic inputs. Striatal projection neurons (SPNs) are a target of both cortical and thalamic projections. Also, different types of

interneurons are targeted by these afferents. The center lateral /parafascicular (CL/Pf) complex, is the main source of thalamostriatal connections, providing only minor inputs to the cerebral cortex. Using whole cell patch clamp recordings in mouse brain slices where both cortical and thalamic inputs were preserved, we compared corticostriatal and thalamostriatal synaptic responses in both classes of striatal projection neurons, direct (dSPNs) and indirect (iSPNs), in fast spiking interneurons (FS), cholinergic interneurons (TAN) and interneurons exhibiting low threshold spikes (LTS). Field stimulation with a concentric bipolar electrode (tip: 50 µm) or illumination of channelrhodopsin-2 (ChR2) delivered to the CL/Pf complex or the cerebral cortex, by transfection with adeno-associated virus containing the double-floxed sequence for ChR2-YFP-CAMKII, were used. ChR2 illumination allowed the exclusive activation of desired neurons or synapses with a blue (473 nm) laser or a LED light. Previous work (Vizcarra-Chacón et al, 2013) showed the complexity of the polysynaptic activation of corticostriatal responses made of several synaptic and voltage-gated currents. Here, we show that polysynaptic suprathreshold responses when stimulating the CL/Pf complex are as complex as corticostriatal responses. Moreover, both thalamic and cortical responses were similar although the differences between dSPNs and iSPNs remain. Similar orthodromic responses were observed after optogenetic cortical or CL/Pf stimulation. Different genres of interneurons were similarly activated by the thalamus or the cortex. We conclude that a great part of striatal synaptic integration depends on the postsynaptic intrinsic properties of striatal neurons and largely independent on the class of afferent (cortical or thalamic). These results also reinforce the view that each neuron class has a particular way to process the same inputs and that striatal output has to be studied in terms of circuitry processing between different neuron classes.

Disclosures: **M.A. Arias:** None. **J. Bargas:** None. **D. Tapia:** None. **E. Galarraga:** None.

Poster

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Title: Alterations in gating of hippocampal and amygdalar inputs to the nucleus accumbens induced by disinhibition of the prefrontal cortex

Authors: ***M. TSE**[1], M. AUGER[1], S. B. FLORESCO[2];
[2]Psychology and Brain Res. Ctr., [1]Univ. British Columbia, Vancouver, BC, Canada

Abstract: Pathophysiological alterations in prefrontal cortex (PFC) GABA transmission have been proposed to underlie various psychiatric disorders. Pharmacological reduction of PFC GABA signalling can produce a variety of cognitive, affective and dopaminergic abnormalities that resemble schizophrenia, including impaired spatial reference memory (mediated by the hippocampus) and aberrant attributions of salience to fear-related stimuli (mediated by the amygdala). Inputs from the PFC, hippocampus and amygdala converge within the nucleus accumbens (NAc), yet the manner in which disinhibitory increases in PFC outflow may affect ventral striatal integration of cognitive and emotional information arising from these temporal lobe inputs remains to be elucidated. We recorded from NAc neurons that received inputs from either the hippocampus or the basolateral amygdala (BLA) in urethane-anesthetized rats. Under basal conditions, stimulation of fimbria/fornix (conveying hippocampal output) and the BLA reliably evoked spike firing in separate populations of NAc neurons. Disinhibition of the PFC via local infusion of the GABA-A antagonist bicuculline (25 or 50ng) attenuated hippocampal-evoked firing in 100% of neurons tested. In contrast, reducing PFC GABA with a lower (25 ng) dose of bicuculline enhanced BLA-evoked firing. In comparison, intra-PFC infusions of a higher, 50 ng dose, revealed two populations of NAc neurons, one (66%) where BLA-evoked firing was suppressed and another (33%) where BLA-evoked activity was unchanged or increased. In neurons that received convergent input from both hippocampal and amygdala inputs, disinhibition of the PFC reduced firing evoked by both inputs. These data suggest that reduced PFC GABA activity may differentially gate mnemonic and emotional signals originating from different regions within the temporal lobes and converging in the NAc. Furthermore, this suggests that perturbations in PFC GABA transmission similar to those that may occur in schizophrenia can lead to altered gating of these inputs to the NAc. This in turn may contribute to impairments in hippocampal-mediated cognitive function and aberrant affective salience attribution and increased anxiety associated with the disorder. Future studies will investigate the potential contribution of increased activation of midbrain dopamine neurons in mediating the gating of hippocampal and amygdala inputs into the NAc, and how PFC disinhibition may induced differential patterns of immediate early gene expression in downstream targets.

Disclosures: **M. Tse:** None. **M. Auger:** None. **S.B. Floresco:** None.

Poster

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IMPULSA03

Title: Action of parvalbumin expressing interneurons in the striatal microcircuit

Authors: *M. DUHNE, E. LARA, A. LAVILLE, J. BARGAS;
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Abstract: The striatum is mainly composed of striatal projection neurons (SPNs). Here, we used optogenetic techniques and dynamic calcium imaging to search into the function of parvalbumin (PV+) expressing interneurons within the striatal microcircuit activity. We injected PV-Cre mice with adeno-associated viral vector: pAAV-EF1a-DIO-hChR2(H134R)-EYFP-WPRE-HGHpA. ChR2 stimulation allowed the exclusive activation of PV+ neurons with a blue (473 nm) laser or a LED light, while recording the activity of dozens of neurons simultaneously with single cell resolution. As previously described, striatal network activity in the presence of micromolar NMDA consisted on the presence of correlated firing or the spontaneous co-activation of several neurons in the same time window (network states). Recurrence, alternation among network states and reverberant behavior following so-called Hebbian sequences were observed (Carrillo-Reid et al. 2008). During the optogenetic activation of PV+ neurons network activity was inhibited by a few milliseconds. Thereafter, a rebound activation of the circuit was noticed together with a resetting of the striatal microcircuit activity: a transient new network state appeared. This new state was composed by previously active and inactive neurons and its appearance disrupted the control Hebbian sequence which was then substituted by a new sequence that lasted for a few minutes before returning to the control sequence. We hypothesize that activation of PV+ neurons causes a temporal switch in a sequence of motor commands to rapidly change or introduce a different motor act or sequence.

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Poster

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Title: Coinciding decreases in discharge rate suggest that spontaneous pauses in firing of external pallidum neurons are network driven

Authors: *E. SCHECHTMAN[1], A. ADLER[2], M. DEFFAINS[3], H. GABBAY[3], S. KATABI[3], A. MIZRAHI[3], H. BERGMAN[3];
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Abstract: The external segment of the globus pallidus (GPe) is one of the core nuclei of the basal ganglia, playing a major role in normal control of behavior and in the pathophysiology of basal ganglia related disorders such as Parkinson's disease. In-vivo, most neurons in the GPe are characterized by high firing rates (50-100 sp/s), interspersed with long (~0.6 s) periods of complete silence termed GPe pauses. Previous physiological studies of single and pairs of GPe neurons have failed to fully disclose the physiological process by which these pauses originate. We examined 1001 simultaneously recorded pairs of high frequency discharge GPe cells recorded from 4 monkeys during task-irrelevant periods, considering the activity in one cell while the other is pausing. We found that pauses (n=137,278 pauses) coincide with a small (0.78±0.136 spikes/s) yet significant reduction in firing rate in other GPe cells. Additionally, we found an increase in the probability of the simultaneously recorded cell to pause during the pause period of the "trigger" cell. Importantly, this increase in the probability to pause at the same time does not account for the reduction in firing rate by itself. Modelling of GPe cells as class 2 excitability neurons (Hodgkin, 1948) with common external inputs can explain our results. We suggest that common inputs decrease GPe discharge rate and lead to a bifurcation phenomenon (pause) in some of the GPe neurons.

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Poster

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Support: Wallenberg Academy Fellowship to G.S

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Title: Synaptic properties of projections from primary somatosensory cortex onto different types of striatal neurons

Authors: *Y. M. JOHANSSON, G. SILBERBERG;
Karolinska Institutet, Stockholm, Sweden

Abstract: Sensory information is highly crucial for the selection and generation of behavior. The striatum constitutes a key component of sensorimotor functions as it integrates information from the motor and somatosensory cortex for generating appropriate movements. Projections from the primary somatosensory cortex (S1) provide sensory information to medium spiny neurons (MSN) and various types of interneurons in the striatal microcircuit. These interneurons exert a powerful control of the MSN, which in turn convey the final output to downstream targets. While the synaptic properties of somatosensory input to MSN are well established, only little is known about the input to the different striatal interneurons. Here, we studied the synaptic properties of somatosensory projections to distinct types of striatal interneurons in Lhx6 mice. We obtained simultaneous whole-cell recordings of MSN and neighboring interneurons in acute striatal slices while selectively activating somatosensory terminals with optogenetics. In the presence of gabazine, monosynaptic responses were recorded following to brief stimulations or trains of stimulations of the presynaptic terminals. We found that light-evoked activation of somatosensory input elicited excitatory postsynaptic potentials (EPSPs) in MSN as well as fast-spiking (FS), low-threshold spiking (LTS) and cholinergic interneurons. The synaptic strength of these connections varied depending on the postsynaptic cell type. FS interneurons received stronger input than simultaneously recorded MSNs whereas both LTS and cholinergic interneurons displayed smaller responses. The dynamics of these cortico-striatal synapses were studied by activating the somatosensory terminals repetitively at different frequencies. Interestingly, activity-dependent depression of synaptic efficacy was more pronounced in MSN than in FS. The response of LTS interneurons was characterized by a comparatively shorter time constant of recovery from depression and by lower release probabilities. Additionally, the

number and conductance of postsynaptic AMPA receptors that were activated upon somatosensory input were assessed for MSN and FS cells. Taken together, these findings indicate that input from S1 elicits cell-type specific responses in the striatum and that both pre- and postsynaptic mechanisms contribute to the unique response pattern of the respective cell types.

Disclosures: Y.M. Johansson: None. **G. Silberberg:** None.

Poster

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Title: Up-states in striatal medium spiny neurons depend on spatial and temporal distribution of cortical input

Authors: *D. B. DORMAN, K. T. BLACKWELL;
Krasnow Inst. For Advanced Study, George Mason, Fairfax, VA

Abstract: Medium spiny neurons (MSNs) of the striatum, by integrating cortical, thalamic, and dopaminergic input, underlie goal-directed learning and habit formation. These neurons exhibit state transitions *in vivo* between a hyperpolarized down-state and a subthreshold depolarized up-state that is driven by spontaneous, asynchronous synaptic activity. Experimental work has shown these MSNs exhibit spike timing dependent plasticity; however, most of those studies were performed in brain slices, in which the MSNs are persistently in the down-state. In contrast, synaptic plasticity during the up-state is not well understood. A critical question underlying MSN plasticity is: how do MSNs respond consistently and selectively to spatially- and temporally-distributed excitatory input? Their ability to respond selectively to a subset of cortical inputs during an up-state may underlie their context-dependence in learning and behavior. Recent experimental work (Plotkin et al., 2011) has shown that temporally aligned synaptic stimulation of ~11 adjacent spines on a distal tertiary dendrite produces a regenerative up-state like depolarization at the soma, which is dependent on NMDA and voltage-gated calcium channels. To address spatiotemporal dependence of the MSN response to cortical input, we developed a

biophysical, multi-compartment MSN model with a branching dendritic structure consisting of primary, secondary, and tertiary branches. Additionally, we explicitly model dendritic spines (density of 0.3/μm) consisting of a spherical head and cylindrical neck. Our model uniquely incorporates complex calcium dynamics including buffers, pumps, and diffusion. We tune the model with experimental data, including IV and IF curves, calcium imaging demonstrating a distance-dependent calcium response to back-propagating action potentials, calcium imaging of spine and dendrite responses to synaptic stimulation, and voltage-clamp investigations of calcium-dependent inactivation of voltage gated calcium channels. We validate the model by reproducing the experimentally-observed upstate-like response to closely aligned synaptic stimulation of distal but not proximal dendritic spines (Plotkin et al., 2011). Using this validated model, we conduct simulation experiments beyond current experimental knowledge to investigate the spatial and temporal dispersion of synaptic stimulation necessary to produce an up-state-like response. In addition, to understand how MSNs may consistently respond to cortical input during repeated behavioral trials, we investigate the sensitivity of calcium elevation in spines to inter-trial variability of cortical input.

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Poster

707. Basal Ganglia: Input Integration

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Topic: D.15. Basal Ganglia

Title: Optogenetic assessment of dynamic input integration in the ventral striatum

Authors: *J. M. BROOKS, P. O'DONNELL;
Pfizer, Cambridge, MA

Abstract: Striatal medium spiny neurons (MSN) serve as a site of convergence for multiple brain regions involved in goal-directed behavior, including the prefrontal cortex (PFC) and hippocampus (HP). These distinct excitatory inputs are believed to differentially influence striatal circuitry in an activity dependent manner. Electrophysiological recordings from anesthetized rats showed that robust PFC stimulation leads to a reduction in ongoing HP-evoked MSN responses, in part, through the recruitment of local inhibitory mechanisms within the ventral striatum (VS). These data indicate that burst-like cortical activity is capable of attenuating weaker, less salient excitatory input within the striatum. Here, we explored the heterosynaptic mechanisms involved in cortical suppression of competing excitatory synaptic

inputs on ventral striatal MSNs. Whole-cell current-clamp recordings were performed from rats receiving bilateral hippocampal injections of a viral vector (AAV) expressing channelrhodopsin 2 (ChR2) under the CamKinase II promoter. Input interactions were assed in VS MSNs through electrical stimulation of PFC fiber tracts and light pulse stimulation of HP inputs expressing ChR2. We have demonstrated that optogenetically evoked HP EPSPs are greatly attenuated after a short latency (50 ms) following burst-like corticostriatal stimulation (5 pulses, 20 Hz, 0.1-0.5 mA) but not a longer (500 ms) delay. Bath application of picrotoxin (100 uM), but not saclofen (2 uM), reduced the magnitude of suppression suggesting inhibitory $GABA_A$, but not $GABA_b$, receptor activation is likely to play a role. As the reduction is not complete, we assessed the role of two signaling molecules which are known to modulate striatal neurotransmission in a retrograde manner. In the VS, endocannabinoid activation of CB1 receptors reduces presynaptic glutamate and GABA release. In addition, subsets of VS MSNs contain dynorphin which, upon release, could decrease glutamate release through activation of presynaptic kappa opioid receptors (KOR). We found that bath application of the CB1 receptor antagonist AM251 (2 uM) enhanced cortical suppression of optically evoked HP responses suggesting the locus of action for AM251 is on inhibitory interneurons. Similar experiments are being conducted using the KOR antagonist nor-BNI. These findings further substantiate the assertion that shifts in VS neuronal activity involve local suppression of competing afferent inputs converging on the same MSN. Furthermore, these data suggest local heterosyanptic suppression involves several signaling pathways.

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Poster

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Université de Bordeaux

Labex Brain (ANR-10-LABX-0043)

Title: Properties of motor cortico-subthalamic neurons: an *in vitro* study

Authors: L. FROUX[1], M. LE BON - JEGO[1], S. MORIN[1], E. NORMAND[2], A. FRICK[3], J. BAUFRETON[1], *A. I. TAUPIGNON[1];
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Abstract: Basal ganglia are a group of interconnected nuclei, that contribute to cortico-cortical loops to control several functions, e.g. voluntary motor behavior. The functions of the basal ganglia are classically thought to rely on the opposite role of two pathways. In this view, the major input stage of the basal ganglia is the striatum. Excitatory corticofugal fibers target two neuron populations in the striatum, forming the divergent direct and indirect pathways. However, corticofugal fibers also target the subthalamic nucleus (STN), a component nucleus, and the sole glutamatergic nucleus in the basal ganglia. The cortico-subthalamic (cortico-STN) connection is also known as the 'hyperdirect pathway'. It is thought to contribute in stopping an already planned action and to be involved in reinforcing the influence of cortex on STN in Parkinson's disease. If subthalamic neuron firing properties are well described, few studies focused on the pre-synaptic side of the hyperdirect pathway, layer 5 pyramidal cortico-STN neurons. The input-output properties of the cortico-STN neurons and the short-term plasticity of the cortico-STN synapses are still unknown. Furthermore, it is not yet understood how STN neurons receive and process cortical input. Using patch-clamp in rodent brain slices combined to two different approaches both relying on stereotaxically-driven viral transfection *in vivo*, we studied (i) the electrophysiological properties of the cortico-STN neurons and (ii) the short-term properties of the cortico-STN synapses. First, we injected a recombinant Rabies-based retrograde virus into the STN to drive the expression of the reporter protein mCherry in cortico-STN neurons. Then, we identified the mCherry-positive neurons in motor cortex and recorded their electrophysiological properties. Second, we injected an anterograde virus expression vector into motor cortex to express the ChR2-mCherry or ChR2-EYFP fusion proteins. Our results in cortex showed that layer 5 pyramidal neurons firing was reliably driven up to 40 Hz using trains of flashes. Furthermore, this approach produced glutamate release from the corticofugal fibers in the STN when we illuminated the internal capsule or the STN with blue light flashes (473 nm). We recorded the resulting excitatory post-synaptic currents in STN neurons and examined the short-term plasticity profile of the motor cortico-STN synapses between 0.05 Hz and 40 Hz. These results shed light on the pre-synaptic properties of the cortico-STN synapses. Together with investigations in the 6-OHDA rat model, these results will help to understand basal ganglia functioning in normal and pathological state.

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Poster

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Title: Distinct cortical GABAergic projecting neurons differentially modulate the activity of striatal neurons

Authors: *S. MELZER[1,2], M. GIL[2], M. MICHAEL[2], H. MONYER[2];
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Abstract: Intricate interactions between cortical areas and striatum are essential for the integration and transformation of sensory inputs and past experiences into value-based decisions and motor outputs. It is long known that excitatory neurons from essentially all cortical areas project to the striatum. Excitatory connections are therefore thought to underlie executive processes in the basal ganglia loop. Using optogenetics combined with *in vitro* electrophysiology and histochemistry, we show here that GABAergic long-range projections constitute a substantial component of cortico-striatal connections. GABAergic projecting neurons are located presumably in all cortical areas and constitute subpopulations of parvalbumin (PV)- and somatostatin (SOM)-expressing neurons. A closer look at the target specificity of GABAergic projections from primary motor cortex revealed that projecting neurons have diverse innervation patterns. While PV-expressing neurons targeted preferentially direct pathway striatal projecting neurons, SOM-expressing neurons were less selective. Additional variability was observed when comparing GABAergic projections from different cortical areas. Our data suggest that cortico-striatal GABAergic neurons are important for the modulation of striatal output and motor activity.

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Title: Cortically induced responses in the basal ganglia through the cortico-striatal neurons

Authors: *H. SANO[1], K. KOBAYASHI[2], S. CHIKEN[1], S. KATO[3], K. KOBAYASHI[3], A. NAMBU[1];
[2]Sec of Viral Vec Dev, [1]Nat Inst. for Physiol Sci., Okazaki, Japan; [3]Dept of Mol Gene, Fukushima Med. Univ., Fukushima, Japan

Abstract: The basal ganglia receive cortical inputs and control voluntary movements. The motor cortex sends afferents to input stations of the basal ganglia, i.e., the striatum and subthalamic nucleus (STN). Both of cortico-striatal and cortico-STN neurons are glutamatergic, and neither specific gene markers nor specific receptors are known. Therefore, it is difficult to selectively manipulate the activity of cortico-striatal or cortico-STN neurons. In the present study, we have applied optogenetics combined with retrograde transport of viral vectors to selectively modulate the activity of cortico-striatal neurons. To induce the selective expression of channelrhodopsin-2 (ChR2) in cortico-striatal neurons, lentiviral vector termed neuron-specific retrograde gene transfer (NeuRet) was injected into the mouse striatum to induce the expression of Cre recombinase in cortico-striatal neurons. Then, adeno-associated viral vector containing a double-floxed inverted open reading frame encoding ChR2 was injected into the motor cortex. The expression of ChR2 is observed in cortico-striatal neurons and laser illumination to the motor cortex induced excitation in these neurons. We gave photostimulation to the motor cortex and recorded neural activity in the basal ganglia. Photostimulation mainly induced biphasic responses composed of inhibition and late excitation in the external segment of the globus pallidus (GPe) and the substantia nigra pars reticulata (SNr), while electrical stimulation to the motor cortex induced triphasic responses composed of early excitation, inhibition and late excitation in these nuclei. These data indicate that inhibition and late excitation in the GPe and SNr evoked by cortical photostimulation are mediated by the cortico-striatal neurons, and that early excitation evoked by electrical stimulation is mediated by other neurons, such as the cortico-STN neurons.

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Poster

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Topic: D.15. Basal Ganglia

Title: Plasticity in collateral connectivity of striatal projection neurons follows learning of a skilled motor task

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Abstract: Neostriatum, the main nucleus of the basal ganglia, participates in motor sequence selection and performance. During acquisition and consolidation of a motor task neostriatal neuronal activity changes dynamically suggesting that changes in synaptic plasticity and synaptic contact reorganization are taking place (Yin et al, 2009). SPNs make up 90% of neostriatum in rodents, they divide in two groups giving origin to parallel output systems the direct (dSPNs) and indirect (iSPNs) pathways of the basal ganglia. Both populations of SPNs connect locally by extensive axon collaterals that exert inhibition on neighboring neurons (Taverna et al, 2008, Lopez-Huerta, et al. 2013). Connections between neurons of either output pathway are important for information processing and final neostriatal output. To test the relevance of these pathways and their synaptic changes following motor skill acquisition has been challenging. In order to address this issue, we trained mice for six days on a single-pellet-retrieval task. We used adenoviral gene transfection of ChR-2 in D1-Cre and A2a-Cre mice. Optogenetic stimulation of dSPNs or iSPNs allowed us selective and widespread activation of each neuronal population. Optical stimulation combined with patch-clamp whole cell recordings of inhibitory postsynaptic currents (IPSCs) were performed in tissue obtained from control and trained mice. We tested collateral connectivity of both spiny neuron populations and evaluated 1) the strength of collateral input from each group of SPN and 2) connectivity rules within the striatal microcircuit under different conditions. Comparison between groups indicates a drop in the number of iSPN to dSPN connections between control and trained groups particularly on the side ipsilateral to the preferred paw with an associated progressive increase in the amplitude of the IPSC. The dSPN to iSPN connections between control and trained animals suffer a smaller decrease and differ less between sides. We are grateful for permission to use the A2a-cre mice from Dr. Alban de Kerchove, Laboratory of Neurophysiology, Université libre de Bruxelles, Belgium.

Changes in synaptic connectivity after learning of skilled motor task

D1-Cre and A2a-Cre mice	Side in rela-tion to pre-ferred paw	% iSPN to dSPN (or iSPN)	Md ± range (pA) IPSC amplitude	% dSPN to iSPN	Md ± range (pA) IPSC ampli¬tude
Control Untrained mice	Not determined	100%	75±15 n=5	50%	63±26 n=6
ExperimentalMice trained in single-pellet-retrieval	Contralateral	89%	57±25 n=8	43%	42±15 n=6
	Ipsilateral	30%	115±43 n=7	33%	66±17 n=6

Disclosures: **V.G. Lopez Huerta:** None. **Y. Nakano:** None. **M. Garcia-Munoz:** None. **G.W. Arbuthnott:** None.

Poster

707. Basal Ganglia: Input Integration

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 707.13/T18

Topic: D.15. Basal Ganglia

Support: NIDA IRP funds

Title: Modulatory role of dopamine D4 receptor on methamphetamine-induced dopamine release

Authors: ***J. BONAVENTURA**[1], C. QUIROZ[1], M. RUBINSTEIN[2], G. TANDA[1], S. FERRÉ[1]; [1]NIDA/NIH, Baltimore, MD; [2]Inst. de Investigaciones en Ingeniería Genética y Biología Molecular, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

Abstract: The human dopamine (DA) D4 receptor gene contains a large number of polymorphisms in its coding sequence. The most extensive is found in the region coding the third intracellular loop of the receptor and consists of a variable number of tandem repeats, in which a 48-bp sequence is repeated from 2 to 11 times. The two most common variants contain four repeats (D4.4) or seven repeats (D4.7), with allelic frequencies of about 60% and 20%, respectively. D4.7 has been consistently associated with low constraint, constituting a risk factor for ADHD and substance use disorder (SUD), but little is known about the underlying

mechanisms. Nevertheless it is generally assumed that D4.7 is “less functional” that D4.4 receptor. D4 receptors are highly expressed by pyramidal cortico-striatal glutamatergic neurons of the prefrontal cortex (PFC), including their striatal terminals. Cortico-striatal neurons -in particular those connecting the medial PFC and the medial Nucleus Accumbens (mNAc) shell- play an important role processing cues and events involved in drug seeking. Cortico-striatal glutamatergic neurotransmission seems to also be involved in the acute effects of some drugs of abuse, particularly THC and methamphetamine (METH). Apart from producing a large increase in DA release, METH has been reported to increase the extracellular concentration of glutamate (GLU) in the NAc by not yet well understood mechanisms. We sought to bring new clues into those mechanisms by using knock-in mice carrying a humanized 7-repeat intracellular loop identical to that found in the human D4.7 variant. Extracellular concentrations of DA and GLU were analyzed in the mNAc shell with *in vivo* microdialysis upon acute administration of METH or cocaine. METH (1 mg/kg, i.p.), but not cocaine (1-10 mg/kg, i.p.), produced a significantly lower increase in NAc DA in D4.7 knock-in mice compared to their WT littermates. Interestingly, METH also increased NAc GLU in D4.7 mice but not in WT littermates. Our hypothesis is that this differential effect on both NAc DA and GLU depends on the reduced inhibitory control of NAc GLU release by D4.7. The reduced DA release would be secondary to an increased GLU-mediated feedback inhibition of VTA DA cells by GABAergic striatal-mesencephalic neurons. This hypothesis will be tested by a new microdialysis-optogenetic approach that allows the measurement of GLU induced by the local selective optogenetic stimulation of the glutamatergic terminals innervating the mNAc shell from the infralimbic cortex. We expect to find a reduced control of GLU release in D4.7 mice compared to WT littermates.

Disclosures: J. Bonaventura: None. **C. Quiroz:** None. **M. Rubinstein:** None. **G. Tanda:** None. **S. Ferré:** None.

Poster

707. Basal Ganglia: Input Integration

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Topic: D.15. Basal Ganglia

Support: NIH Intramural Research Program

Title: Functional anatomy of the infralimbic-accumbens pathway: a microdialysis-optogenetic approach

Authors: *C. R. QUIROZ, M. ORRU, W. REA, A. CIUDAD, S. FERRE;
Medications Discovery Br., NIDA, IRP, NIH, DHHS, Baltimore, MD

Abstract: The glutamatergic pathway that connects the medial prefrontal cortex (MPC) with the nucleus accumbens (NAc) has received substantial attention due to its involvement in the acquisition and elicitation of approach or withdrawal behaviors. It is generally assumed that the prelimbic (PL) and infralimbic (IL) cortices are the corresponding cortical areas projecting to the NAc core and NAc shell. However, there is evidence for a predominant latero-medial striatal functional compartmentalization, dependent on differential connectivity. In this way NAc shell can be functionally divided into lateral and medial portions. The medial portion of the NAc shell (mNAc shell) seems to be preferentially innervated by the IL cortex and by the most caudal and medial part of the ventral tegmental area. Yet, a clear functional distinction exists between the rostral and caudal portions of the mNAc shell, with differential involvement in appetitive and fearful behaviors, respectively. We initially aimed at establishing a neurochemical correlate of the differential function between the rostral and caudal portions of the mNAc shell. Combining electrical stimulation with immunohistochemistry (P-ERK1/2) and *in vivo* microdialysis (glutamate and dopamine release) we found evidence for a selective functional connection between the IL cortex and the caudal (2/3) but not more rostral (1/3) portion of the mNAc shell. Thus, the IL cortex innervates the whole “fear”-related and a fraction of the “desire”-related portion of the mNAc shell. Therefore, the established “anatomical” separation between PL and IL cortices should be changed to a more functional demarcation. As previously reported for other areas of the MPC, IL electrical stimulation was associated with glutamate and also dopamine release in the mNAc shell. The introduction of a new microdialysis-optogenetic approach allowed solving the controversy about the role of MPC afferents to the VTA versus local activation of glutamate receptors localized in NAc dopaminergic terminals in the elicitation of dopamine release in the NAc induced by MPC stimulation. Optogenetic stimulation of IL-mNAc shell terminals elicited glutamate and dopamine release, which were completely counteracted by the local perfusion of a blocker of glutamate release (an adenosine A2A receptor antagonist). On the other hand, the A2A receptor antagonist counteracted glutamate but only partially dopamine release in the mNAc shell induced by IL electrical stimulation. The results indicate that the IL cortex controls the extracellular levels of dopamine in the mNAc shell by means of both the direct IL-NAc pathway and an indirect IL-VTA-NAc pathway.

Disclosures: C.R. Quiroz: None. **M. Orru:** None. **W. Rea:** None. **A. Ciudad:** None. **S. Ferre:** None.

Poster

707. Basal Ganglia: Input Integration

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Topic: D.15. Basal Ganglia

Support: ERC starting grant

Wallenberg Academy Fellowship

Title: Corticostriatal pathways underlying bilateral sensory integration in the mouse striatum

Authors: *R. REIG, G. SILBERBERG;
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Abstract: Rodents use their whiskers to actively explore their surroundings, simultaneously engaging sensory and motor processes. Cortical somatosensory and motor areas provide glutamatergic input to the basal ganglia via corticostriatal pathways. Individual projection neurons and interneurons in dorsal striatum integrate tactile sensory input from both ipsi- and contralateral whiskers (Reig & Silberberg 2014). The pathways underlying this integration are, however, largely unknown. The aim of this study is to understand the sensory pathways underlying the ipsi- and contralateral tactile responses in striatal neurons. To that end, we used simultaneous *in vivo* whole-cell patch-clamp recordings in striatum as well as extracellular recordings in the neocortex of anesthetized mice. In order to investigate corticostriatal projections, we recorded sensory responses before and after selectively silencing different cortical areas by local TTX injections. Inactivation of the ipsilateral cortical barrel field resulted in strong bilateral decrease of sensory responses. Inactivation of the contralateral barrel field did not affect the response to contralateral whisker deflection but reduced the response to ipsilateral whisker by more than 70%. Inactivation of motor cortex affected only later (> 200 ms) components of the response but not the early stage. We showed that whereas responses to contralateral whisker stimulation are mediated by ipsilateral corticostriatal projections from the barrel cortex, responses to ipsilateral whisker stimulation are mediated mainly by parallel cortico-cortical pathways (mainly cross callosal projections between barrel fields), rather than a direct corticostriatal projection from the contralateral barrel field.

Disclosures: R. Reig: None. **G. Silberberg:** None.

Poster

707. Basal Ganglia: Input Integration

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Topic: F.02. Animal Cognition and Behavior

Support: Grant-in-Aid for Scientific Research on Innovative Areas from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (26112002)

Title: Thalamostriatal system controls selection and flexibility of motor actions

Authors: *K. KOBAYASHI[1], S. KATO[1], M. OKAMOTO[2], S. EIFUKU[2];
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Abstract: The basal ganglia circuitry plays important functions in the appropriate selection and flexible switching of behaviors. The dorsal striatum is a principle structure of the basal ganglia circuitry and mediates learning processes of instrumental motor actions. Anatomically, the dorsal striatum receives glutamatergic excitatory afferents from many cortical areas and the intralaminar thalamic nuclear groups, including the central lateral nucleus (CL) and parafascicular nucleus (PF) as well as dopaminergic projections from the ventral midbrain. In rodents, the CL and PF are localized in the rostral and caudal regions of the intralaminar thalamic nuclear groups, which project directly to medium spiny neurons and interneurons in the striatum. We reported that the PF-derived thalamostriatal pathway is important for the acquisition and performance of sensory discrimination task in mice. To address the roles of the CL-derived thalamostriatal system in learning processes, we performed the selective neural pathway targeting by combining a highly efficient retrograde gene transfer (HiRet) vector and immunotoxin (IT)-mediated cell targeting. Human interleukin-2 receptor α-subunit (IL-2Rα), a receptor molecule for the recombinant IT, was expressed in the neurons innervating the striatum in mice by using the HiRet vector. Treatment with IT into the CL in the vector-injected mice induced a selective, efficient elimination of the thalamostriatal pathway, resulting in the lack of electrophysiological responses elicited by the CL stimulation. The elimination of CL-derived thalamostriatal pathway did not alter the acquisition of visual discrimination, whereas it impaired transiently the accuracy of motor responses and lengthened the response time. In addition, the pathway elimination disturbed the reversal of response discrimination and the set shifting from visual discrimination to response discrimination. These results suggest that the thalamostriatal system originating from the CL predominantly regulates selection and flexibility of motor actions, which requires the function of the prefrontal cortex-basal ganglia circuitry.

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Poster

708. Basal Ganglia Anatomy and Physiology

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Topic: D.15. Basal Ganglia

Support: Santos Dumont Institute

AASDAP

FINEP

Incemaq (Programa INCTs do CNPq/MCT)

FAPERN

CAPES

CNPq

Title: Neuronal activity in the marmoset basal ganglia thalamocortical circuit during rest and spontaneous locomotion

Authors: *M. F. ARAUJO[1], R. MOIOLI[2], E. MORYA[2];
[1]Edmond and Lily Safra Intl. Inst. of Neurosci., Inst. Santos Dumont, Macaíba, Brazil; [2]Edmond and Lily Safra Intl. Inst. of Neurosci., Santos Dumont Inst., Macaíba, Brazil

Abstract: The basal ganglia thalamocortical pathway is involved in the regulation of motor function. Dysfunction in this pathway is related to a number of movement disorders such as Parkinson and Huntigton diseases. The description of the neuronal dynamics and firing patterns in this circuit is therefore essential to better understand the patophysiology of such disorders. The present work aimed at describing the activity of neurons in the basal ganglia thalamocortical circuit in the common marmoset. Two microelectrode arrays of 32 microelectrodes were bilaterally implanted in a female marmoset. The arrays were designed to to simultaneously record from the motor cortex and 3 basal ganglia nuclei: putamen, internal globus pallidus and external globus pallidus. Local field potentials (LFPs) and single and multi-unit activity were recorded from all the implanted structures while the animal freely moved inside a plexiglass box (45 x 45 cm). Her behavior was recorded by 2 video cameras positioned at the top and at the front of the box. Each experimental session lasted 10 min. All procedures were approved by the Institution's Ethics Comittee for Animal Use (Protocol 11/2011). On average, 33.9 units were recorded at each of the 8 experimental sessions. Rest and spontaneous locomotion periods were extracted from the video and synchronized with spike data. We describe and compare the neuronal activity patterns found in the 4 recorded structures during these 2 conditions. These

results contribute to the understanding of the basal ganglia thalamocortical circuit functioning during rest and spontaneous movement execution and provides grounds for future studies using common marmosets as models to study movement disorders.

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Poster

708. Basal Ganglia Anatomy and Physiology

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Topic: D.15. Basal Ganglia

Support: USPHS NIH NS-23805

Title: Exclusivity in the connections of the rostral division of the zona incerta

Authors: *D. S. ZAHM, M. T. DESTA;
Pharmacol. and Physiological Sci., St. Louis Univ. Sch. of Med., Saint Louis, MO

Abstract: The zona incerta (ZI) in the rat extends from the dorsolateralmost part of the lateral hypothalamus over the entopeduncular and subthalamic nuclei (EPN and STN) and into the interstice between the internal capsule/cerebral peduncle and basal thalamus from about the level of the EPN to the rostral midbrain. It comprises rostral, caudal and middle divisions, the latter having dorsal and ventral parts. Diverse functions in four categories - visceral response, arousal, attention and postural/locomotion - have been attributed to ZI and, consistent with this, its widespread and diverse connections are reported to involve nearly all part of brain (Mitrofanis, Neuroscience 130:1-5, 2005). Thus, we were surprised in preparing a recent paper on the connections of the midbrain dopaminergic complex (JCN 519:3159-88, 2011) to observe that the density of outputs from the rostral (r) division of ZI drops abruptly to negligible precisely at the dorsal border of the ventral tegmental area, and that ZIr outputs also are few among the dopamine neurons of the substantia nigra compacta and retrorubral field. This effect was more striking in subsequently prepared cases showing anterogradely transported cholera toxin β subunit (Ctβ), which, compared to other tracers, provides minimal definition of axons but a better estimation of projection density. In contrast, projections to numerous other brainstem structures including the mesopontine reticular formation, dorsolateral periaqueductal gray and deep superior colliculus, and thalamic nuclei, including ventral and intralaminar, were exceedingly dense. Ctβ-injected preparations also sensitively demonstrated the afferent projections of ZIr, which, consistent with the cited review, we found to be widespread and

numerous. However, we were again surprised that several basal ganglia structures including the caudate-putamen, accumbens, ventral pallidum, globus pallidus and subthalamic nucleus exhibited zero retrogradely labeled neurons following injections of Ctβ into the ZIr, whereas the related EPN and substantia nigra reticulata had moderate numbers of retrogradely labeled neurons, as did, interestingly, central extended amygdala structures, including the central amygdaloid nucleus, sublenticular region and bed nucleus of stria terminalis, which, actually, exhibited moderate to strong retrograde and anterograde labeling following by ZIr injections of Ctβ. Other ZI divisions remain to be addressed in this way, but, nonetheless, this enigmatic anatomy should have interesting functional implications, particularly in view of recent keen interest in ZI as a target for deep brain stimulation in Parkinson's disease.

Disclosures: D.S. Zahm: None. **M.T. Desta:** None.

Poster

708. Basal Ganglia Anatomy and Physiology

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Topic: D.15. Basal Ganglia

Support: NIH P01 NS044393

NIH P30 NS076405

Title: Primary motor cortex leads globus pallidus in the encoding of kinematics in rhesus macaques

Authors: P. J. RICE[1], B. PASQUEREAU[2], O. DRINKWATER[3], *R. S. TURNER[2];
[1]Univ. of Washington, Seattle, WA; [2]Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA;
[3]Weill Cornell Med. Col., New York City, NY

Abstract: Some theories propose that the basal ganglia (BG) plays a role in early processing of planned movement (requiring that activity in the BG leads the cortex in the encoding of movement kinematics), whereas others suggest a role in feedback control (i.e. the encoding of kinematics appears earlier in cortex than in the BG). To disentangle these potential functions, we compared the timing and strength of kinematic-related signals encoded in the spiking activity of neurons in the globus pallidus (GP) and primary motor cortex (M1). Single-unit activities were recorded in four rhesus monkeys trained to perform an extended series of multi-joint arm movements. A total of 364 cortical neurons and 554 pallidal neurons were collected across the

four animals. To determine the latency of kinematic encoding three independent analytic methods were applied. In the first, the averaged movement-related activities around the time of movement initiation were used to determine response onset latency for each cell recorded within the two structures. In the second, a generalized linear model (GLM) regression analysis was performed on unit activity around the time of the first movement to determine the timing and strength of encoding of the observed kinematics (including direction, maximum speed, extent, duration, and reaction time). The third method applied a GLM to study the temporal relationship between neuronal activity and the ongoing series of movements. This model estimated the degree to which spike rate encoded continuous measures of hand position, velocity, acceleration, and speed at a temporal offset τ, thereby allowing an estimation of the lead or lag between spike firing and the observed kinematics. For all three methods, an ROC analysis was applied to compare latencies between the two populations. The first analysis found no difference in response onset latencies (median M1: -121ms, GP: -110ms; AUC=0.53 P=0.13). The encoding of kinematics, however, appeared earlier in M1 than in GP both around the time of movement initiation (second method; median M1: -89ms, GP: -20ms; AUC=0.66 $P<0.001$) and also during ongoing movement (third method; median τ M1: -100ms, GP: -20ms; AUC=0.71 $P<0.001$). While our results do not support a role of the BG in generating or selecting the motor command, they do suggest an involvement in the monitoring or evaluation of motor performance.

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Poster

708. Basal Ganglia Anatomy and Physiology

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Topic: D.15. Basal Ganglia

Support: NS034865 to JMT

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Title: Loss of neuropilin 2 induces aberrant corticostriatal circuit activity and impairs goal-directed instrumental behavior in mice

Authors: M. W. SHIFLETT[1], M. ASSOUS[2], E. MARTINEZ[3], E. CHOE[3], J. M. TEPPER[2], *T. S. TRAN[3];
[1]Psychology, [2]Ctr. For Mol. And Behavioral Neurosci., [3]Biol. Sci., Rutgers Univ., Newark, NJ

Abstract: Neuropilin 2 is an obligate binding partner to class 3 secreted semaphorins (Sema3s) and has been demonstrated to play an important role in dendritic morphogenesis and synapse refinement in the mammalian nervous system. Our previous findings show Semaphorin 3F (Sema3F) acting through the Neuropilin 2/Plexin-A3 (Nrp2/PlexA3) holoreceptor complex signals *in vivo* to restrain layer 5 cortical neuron dendritic spine morphogenesis. Loss of semaphorin signaling produces exuberant spines on apical dendrites of Layer 5 cortical pyramidal neurons that project axons to the striatum. The effects of Neuropilin 2 signaling on corticostriatal circuit function are unknown. Using mice that harbor a mutation in the locus encoding neuropilin 2 (Nrp2), we investigated cortical synaptic input as well as performance in an instrumental learning task known to depend on corticostriatal circuit function. Whole cell recordings from corticostriatal coronal brain slices revealed that Nrp2-/- mutants displayed impaired pair pulse ratio compared to wild type (WT) littermate controls. In addition, striatal spiny projection neurons in Nrp2-/- mutant mice showed significantly higher excitability in terms of rheobase current, input resistance, and firing frequency in comparison with the WT littermates. These results suggest both a presynaptic and a postsynaptic impairment. Furthermore, in the instrumental learning task, Nrp2-/- mutants mice showed insensitivity to changes in outcome value when compared to age-matched controls, suggesting their behavior was not goal-directed but rather was controlled by antecedent stimuli. Collectively, these results coupled with previous cellular, molecular and electrophysiological findings suggest that loss of Nrp2 may induce aberrant activity within the corticostriatal pathway giving rise to behavioral and cognitive impairments. This work was supported, in part, by NS034865 to JMT and the Charles and Johanna Busch Biomedical Fund to TST.

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Poster

708. Basal Ganglia Anatomy and Physiology

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Support: CHDI Foundation

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Title: Dysfunction of subthalamic nucleus neurons in mouse models of Huntington's disease

Authors: *J. F. ATHERTON[1], E. MCIVER[1], V. BEAUMONT[2], D. J. SURMEIER[1], M. D. BEVAN[1];
[1]Physiol., Northwestern Univ., Chicago, IL; [2]CHDI Fndn., Los Angeles, CA

Abstract: Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an expansion of a CAG repeat within the huntingtin (htt) gene. In its early stages HD leads to striatal degeneration, with profound effects on basal ganglia circuitry. The BACHD transgenic and Q175 knock-in mouse models of HD, which express full length human mutant htt with 97 and ~179 CAG repeats respectively, are associated with abnormal intrinsic and synaptic properties in cortical and striatal neurons together with neuronal degeneration and behavioral abnormalities. The subthalamic nucleus (STN) is a key element of the basal ganglia, but its properties are relatively uncharacterized in these models. Thus, the aim of this project was to determine whether STN neurons exhibit alterations that may contribute to abnormal behavior. The frequency and precision of autonomous activity of STN neurons in brain slices from BACHD and Q175 mice were compared with wild type (WT) littermate controls at <2 and 6 months for BACHD and at 6 months for Q175. At 2 and 6 months the firing frequency of BACHD and Q175 neurons was reduced and the proportion of inactive neurons increased. Disrupted firing in BACHD was rescued to WT levels by application of the KATP channel antagonist glibenclamide or decomposition of H2O2 by catalase. When co-applied, each occluded the other, suggesting that H2O2 activates KATP channels. Assessment of mitochondrial redox state using 2-photon imaging of roGFP confirmed increased oxidation in BACHD. In BACHD, NMDA receptor (NMDAR)-mediated mEPSCs exhibited prolonged decay times at both <2 and 6 months. Blockade of the excitatory amino acid transporters EAAT1/GLAST and EAAT2/GLT-1 with TFB-TBOA prolonged decay times of NMDAR-mediated EPSCs elicited by optogenetic stimulation of M1 motor cortical axons in WT but not in 6-month-old BACHD, suggesting that dysfunctional glutamate transport may result in excessive NMDAR activation. NMDAR activation can activate KATP channels through NO signaling, implying that abnormal NMDAR activation could underlie firing disruption. Indeed, incubation of WT slices in NMDA for 60 minutes also produced a persistent KATP channel-dependent disruption in firing. Furthermore, application of the NOS blocker L-NAME partially rescued firing in BACHD. Together, these data suggest that disrupted autonomous activity in these models may be triggered by abnormal NMDAR function leading to increased NO production, mitochondrial dysfunction and KATP channel activation.

Disclosures: **J.F. Atherton:** A. Employment/Salary (full or part-time):; Northwestern University. **E. McIver:** A. Employment/Salary (full or part-time):; Northwestern University. **V. Beaumont:** A. Employment/Salary (full or part-time):; CHDI Foundation. **D.J. Surmeier:** A. Employment/Salary (full or part-time):; Northwestern University. **M.D. Bevan:** A. Employment/Salary (full or part-time):; Northwestern University.

Poster

708. Basal Ganglia Anatomy and Physiology

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EUROPEAN RESEARCH COUNCIL (ERC-2013-CoG-615699_NEUROKINEMATIKS, D.R.)

Title: A new task to characterize striatal mechanisms for running control in mice

Authors: C. SALES-CARBONELL, L. KHALKI, *D. M. ROBBE;
Inst. de Neurobiologie de la Méditerranée, Marseille, France

Abstract: The exact role of the basal ganglia in motor control is still highly debated. D1- and D2-positive medium spiny neurons (MSN) of the dorsal striatum form respectively, the direct and indirect pathways of the basal ganglia. These two neuronal populations are classically though to play antagonistic roles in motor control. D1-MSN activation would initiate movement while D2-MSN would contribute to suppression of unwanted actions. Still, the specific contribution of these two pathways in a motor task that requires both active initiation and suppression of movements is still unresolved. Here we developed a new head-fixed behavioral task in which mice are trained to run a fixed distance upon presentation of a simple stimulus in order to obtain a liquid reward. Importantly, upon successful completion of a run, the presentation of a new stimulus is conditioned on the animal being completely immobile for a few seconds and this at a fixed time interval after reward delivery. Once fully trained, mice develop a stereotyped running sequence during which they successively accelerate, run at steady speed, decelerate and completely stop running. Recording of spiking activity was performed in the dorsal striatum of trained mice using silicon probes. We found that a majority of units had their activity strongly modulated at different phases of the task both during running and immobility period. Ongoing statistical analyzes and experiments using optogenetic-based identification of D1 and D2 MSN will reveal the specific contribution of the two populations of MSN in the execution of the distinct phases of our motor task.

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Poster

708. Basal Ganglia Anatomy and Physiology

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Title: Plasticity of motor cortex-subthalamic nucleus transmission in experimental Parkinson's disease

Authors: *H.-Y. CHU[1], M. D. BEVAN[2];
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Abstract: The basal ganglia are a group of subcortical brain nuclei critical for voluntary movement and a major site of dysfunction in movement disorders like Parkinson's disease (PD). To determine how the movement suppressing hyperdirect pathway from the motor cortex (MC) to the subthalamic nucleus (STN) is altered in experimental PD, we interrogated *ex vivo* brain slices derived from 6-hydroxydopamine- or vehicle-injected mice using electrophysiological, optogenetic and immunohistochemical approaches. The properties of MC-STN transmission were significantly altered by the chronic loss of dopamine. Thus, 1) the amplitude of maximal optogenetically stimulated MC-STN AMPA receptor (R)-mediated transmission was reduced by ~70%; 2) the amplitude and frequency of miniature MC-STN transmission, optogenetically evoked in the presence of Sr^{2+}, were reduced by ~15% and ~60% respectively; 3) MC-STN AMPAR-mediated EPSCs exhibited greater inward rectification and sensitivity to 1-naphthyl acetyl spermine trihydrochloride, a selective GluA2 subunit-deficient AMPAR antagonist, indicating an increase in the expression of Ca^{2+} permeable AMPARs; 4) the ratio of NMDAR:AMPAR MC-STN transmission was slightly decreased; 5) the density of vesicle glutamate transporter 1 expressing (cortex-STN) axon terminals was reduced by ~40%. The structural and morphological changes associated with dopamine depletion-induced plasticity of MC-STN synaptic transmission are being investigated further through confocal and 2-photon imaging. Together these data suggest that excessive cortical patterning of the STN in PD is not due to an increase in the strength of MC-STN transmission.

Disclosures: H. Chu: None. **M.D. Bevan:** None.

Poster

708. Basal Ganglia Anatomy and Physiology

Location: Hall A

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Program#/Poster#: 708.08/U9

Topic: D.15. Basal Ganglia

Support: NIH grant R01 DA038890

Office of Naval Research Grant MURI N00014-10-1-0198

Title: Calcium dynamics predicts direction of synaptic plasticity in striatal spiny projection neurons

Authors: *J. JEDRZEJEWSKA-SZMEK[1], S. DAMORADAN[2], D. B. DORMAN[2], K. T. BLACKWELL[3];
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Abstract: The striatum is a major site of learning and memory formation for both sequence learning and habit formation. One of the mechanisms used by the brain for memory storage is synaptic plasticity -- the long-lasting, activity dependent change in synaptic strength. All forms of synaptic plasticity require an elevation in intracellular calcium, and a common hypothesis is that the amplitude and duration of calcium transients can determine the direction of synaptic plasticity. The utility of this hypothesis in the striatum is unclear in part because dopamine is required by striatal plasticity and because of the diversity in stimulation paradigms. To test whether calcium can predict plasticity direction, we developed a calcium based plasticity-rule using a model MSN with sophisticated calcium dynamics including calcium diffusion, buffering, and pump extrusion. We utilize three spike-timing dependent plasticity (STDP) induction paradigms, in which postsynaptic potentials are paired withe precisely timed action potentials. Experimentally, the timing of such pairing determines whether potentiation or depression will occur. Our simulations show that, despite the variation in calcium for different protocols, a single, calcium-based weight change rule (plasticity rule) can explain the change in synaptic weights for all three STDP paradigms. Additional simulations show that the plasticity rule correctly predicts that long-term potentiation (LTP) elicited by forward pairing (but not long-term depression (LTD) induced by backward pairing) is blocked when NMDA receptors are blocked. In addition, the plasticity rule correctly predicts that blocking L-type channels does not influence LTP induced by forward pairing, but blocks LTD induced by backward pairing. Further simulations will explore the role of dopamine by implementing synaptic and ionic channel changes produced by D1 and D2 receptors. Elucidating the mechanisms underlying synaptic plasticity, especially the role and interplay of calcium and dopamine, will allow for better understanding mechanisms of memory storage in health and disease.

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Poster

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Title: Simultaneous functional imaging and neurochemical recording during subthalamic nucleus deep brain stimulation

Authors: ***E. K. ROSS**[1,2], H.-K. MIN[2], M. SETTELL[2], A. MCCONICO[2], S. CHO[2], C. BLAHA[2], S. CHANG[2], K. BENNET[2], K. LEE[2];
[1]Neurosci., Mayo Clin. Col. of Med., Rochester, MN; [2]Mayo Clin., Rochester, MN

Abstract: Introduction: Deep brain stimulation (DBS) within the basal ganglia complex is an effective neurosurgical approach to treat motor symptoms associated with Parkinson's disease (PD). Over 100,000 patients with neurologic and neuropsychiatric disorders have been successfully implanted with DBS systems to date, though the mechanisms still remain poorly understood. It is therefore a common critical goal in stereotactic and functional neurosurgery to elucidate the mechanisms of DBS in order to improve patient care. Here, we set out to address this issue by combining two powerful technologies, functional magnetic resonance imaging (fMRI) and *in vivo* neurochemical monitoring to investigate subthalamic nucleus (STN) DBS-mediated activation of basal ganglia network circuitry. Methods: MR image-guided stereotactic surgery was performed to deliver a chronic mini-DBS electrode to STN in three anesthetized rhesus macaques. 3D functional anatomical data were obtained from STN DBS-evoked blood oxygen level dependent (BOLD) increases during fMRI. Placement of the carbon fiber microelectrode (CFM) was based on the maximum intensity voxel within the respective activation cluster in sub-regions of the basal ganglia. For fast-scan cyclic voltammetry (FSCV), we used wireless instantaneous neurochemical sensing (WINCS Harmoni) system to apply a pyramidal voltage waveform (-0.4V to 1.45V) to the CFM with respect to an Ag/AgCl reference

electrode. All study procedures were compliant with the National Institutes of Health Guidelines for Animal Research and approved by Mayo Clinic IACUC. Results: STN DBS resulted in significant BOLD increases in basal ganglia and motor circuitry (FDR < 0.001), including ipsilateral motor cortex, cingulate gyrus, putamen, caudate nucleus, premotor cortex, and contralateral cerebellum. Additionally, we found stimulation time-locked dopamine release in both caudate and putamen, correlating with the maximum intensity voxel in each activation cluster based on the functional activation map seen in the fMRI. Conclusions: Here, we correlate STN DBS-evoked neurotransmitter release with simultaneous fMRI in the same sub-regions. The simultaneous combination of fMRI and electrochemistry offer a new and exciting approach that provides complementary anatomical mapping and neurochemical monitoring implicated in the therapeutic actions of STN DBS. Importantly, the STN DBS evoked dopamine release may be an important mechanism by which DBS improves PD symptomatology. Acknowledgements: This work was supported in part by The Grainger Foundation and NIH (R01 NS70872 and RO1 NS75013 awarded to KHL).

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Poster

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Title: Beta oscillations between the subthalamic nucleus and substantia nigra pars reticulata during automatic and voluntary movement

Authors: *J. J. JANTZ, M. WATANABE, R. LEVY, D. P. MUNOZ;
Queen's Univ., Kingston, ON, Canada

Abstract: The basal ganglia network (BG) is implicated in switching between automatic and voluntary saccadic eye movements, and can influence saccade initiation via output to the superior colliculus and frontal eye fields. We provide complimentary neurophysiological

evidence by which this may occur, by examining changes in local field potential oscillation and coherence between simultaneously recorded electrodes in the subthalamic nucleus (STN; BG input) and substantia nigra pars reticulata (SNr; BG output). Two monkeys performed an interleaved pro- (look toward) and anti-saccade (look away) task, to recruit automatic and voluntary control, while STN and SNr activity was recorded (n = 15 acute electrode pairs, 1500+ trials per pair). First, we found that STN and SNr coherence was strikingly decreased from baseline in beta band frequencies (15-30 Hz) during the peri-saccadic period in pro- and anti-tasks. Second, we found a strong relationship between beta power and saccade latencies, by grouping trials according to high or low beta band power in either the STN or SNr. During the anti-saccade task, both STN and SNr beta power was inversely correlated with saccade initiation. In stark contrast, STN beta power had no effect on pro-saccade initiation, while SNr beta power was positively correlated with pro-saccade initiation. Overall, we found that STN and SNr beta power had the same effect during anti-saccades, but an opposite effect during pro-saccades. Local field potential recordings reflect the summation of local electrical fields, and an increase in oscillatory power reflects increased synchrony in local neuronal networks. Therefore, we suggest these differences may be attributable to changes in BG networked activity during the suppression of reflexive responses and volitional execution of saccades.

Disclosures: **J.J. Jantz:** None. **M. Watanabe:** None. **R. Levy:** None. **D.P. Munoz:** None.

Poster

708. Basal Ganglia Anatomy and Physiology

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CHDI

Title: Chemogenetic restoration of autonomous subthalamic nucleus activity ameliorates motor deficits in experimental Parkinson's disease

Authors: *E. MCIVER, J. ATHERTON, D. SURMEIER, M. D. BEVAN;
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Abstract: Parkinson's disease (PD) is associated with the degeneration of substantia nigra dopamine neurons, the development of abnormal, synchronous, rhythmic activity in the cortico-basal ganglia-thalamo-cortical circuit, and impaired movement. The origin of this abnormal activity pattern and its relationship to motor dysfunction remain poorly understood. However, the slow emergence of abnormal activity days to weeks after loss of dopamine in the 6-hydroxydopamine (6-OHDA) model of PD implies an important role for plasticity. The subthalamic nucleus (STN) occupies a key position in this circuit, at the intersection of the movement-inhibiting indirect and hyperdirect pathways. We hypothesized that the autonomous activity of STN neurons normally decorrelates indirect and hyperdirect pathway activity and that loss of autonomous firing following dopamine depletion promotes abnormal activity and motor dysfunction. 2-3 weeks after 6-OHDA treatment, *ex vivo* brain slice recordings confirmed that the autonomous firing of STN neurons was profoundly disrupted (sham: 10.2, 4.9-15.1 Hz, 12.6% inactive; 6-OHDA: 2.3, 0-7.4 Hz, 37.5% inactive; median, interquartile range). Disrupted activity was associated with an elevation of reactive oxygen species and was rescued by antagonism of ATP-sensitive potassium (KATP) channels. Activity disruption may be triggered by excessive activation of STN NMDA receptors (Rs) *in vivo*. Indeed, persistent KATP channel-dependent disrupted firing was reproduced *ex vivo* in control tissue by pre-incubation in 25 μM NMDA for 1 hour prior to recording. Furthermore, knockdown of STN NMDARs *in vivo* in 6-OHDA-treated mice prevented autonomous firing disruption and also ameliorated motor dysfunction. These data suggest that an NMDAR-triggered increase in whole-cell KATP channel conductance disrupts intrinsic STN firing in experimental PD and that preventing this disruption may be therapeutic. We next employed a chemogenetic approach to restore intrinsic STN activity in 6-OHDA-treated mice. DREADD activation restored autonomous STN firing *ex vivo* to control levels and reversed motor dysfunction *in vivo*. Together, these findings suggest restoration of intrinsic STN activity as a novel therapeutic approach in PD.

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Poster

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Topic: D.15. Basal Ganglia

Title: Localization of glycinergic neurons in the rodent thalamus

Authors: *P. LOZANO[1], M. PANDO[2], M. MIRANDA[2];
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Abstract: Localization of Glycinergic Neurons in the Rodent Thalamus The thalamus is considered a critical hub integrating a complex array of motor and sensory functions. The detailed anatomy of some neuronal networks, local circuits, inputs from and to the brain stem and cortex are well known. While the general topography is well understood, the position and contribution of glycinergic networks to thalamic functions need to be elucidated. Recent published data and our preliminary studies suggest the presence of glycinergic neurons in the thalamus, whether this immunoreactivity corresponds to glia cells or neurons is something that remains to be investigated. To address this question, we have stained mouse brain sections with anti-glycine transporter 1 antibody and detected strong immunoreactivity throughout the nuclei of the thalamus. The presence of GlyT1 was confirmed by western blotting. Further staining suggests co-localization of GlyT1 with the neuronal marker MAP2 and excluded from GFAP staining, suggesting expression in neuron. Future studies will focus in the characterization of these cells by delivery of the retrograde and anterograde tracers Fluoro-Gold and Fluoro-Ruby, respectively, into the different thalamic nuclei. These experiments should shed light into the glycinergic network in the thalamus. Understanding the neural pathways containing glycine transporters will allow us to have a better knowledge about the inhibitory inputs in the control of sensory-motor integration.

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Poster

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Topic: D.15. Basal Ganglia

Title: Movement modulates phase-locking between neuronal discharge in human STN and cortical oscillations

Authors: *W. J. LIPSKI[1], R. S. TURNER[2], D. J. CRAMMOND[1], E. D. KONDYLIS[1], A. ALHOURANI[1], M. J. RANDAZZO[1], M. RICHARDSON[1];
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Abstract: Deficits in the initiation and control of movement account for many symptoms in Parkinson's disease (PD), including akinesia, bradykinesia, freezing, rigidity and tremor. The

underlying etiology in PD involves the degeneration of the dopaminergic nigrostriatal pathway, which results in increased activity in the subthalamic nucleus (STN). This pathological hyper-activity of STN, in turn, suppresses thalamocortical activity via the indirect pathway, and is thought to result in hypo-active movement deficits such as akinesia, bradykinesia, rigidity, and freezing for which deep brain stimulation (DBS) of the STN is an effective therapy. Recent findings implicate the interactions between sensorimotor cortex and the STN in the pathophysiology of PD, which may contribute to the generation of aberrant cortical-basal ganglia activity that disrupt movement control. However, the physiology of these interactions is not understood. In order to elucidate the role of cortico-subthalamic interactions in movement control, we recorded single neuron (spike) and local field potential (LFP) activity in the STN simultaneously with ECoG activity from sensorimotor cortex of PD patients undergoing STN DBS electrode implantation. Eight subjects performed a bimanual grip force reaction time task intra-operatively during data recording. In 10 of 28 STN neurons recorded from 8 patients, the discharge of single STN neurons was phase-locked to ECoG oscillations in sensorimotor cortex, within the beta (12-30 Hz; N=4) and theta (4-8 Hz; N=6) frequency bands. In a subset of 3 STN neurons showing phase locking, the strength of coupling was modulated during movement. For ipsilateral movements, phase locking, measured one second after grip force onset, was enhanced , while for contralateral movements phase locking was suppressed, relative to pre-movement baseline. Thus, STN neuronal activity became de-synchronized from ECoG oscillations during contralateral movement. Furthermore, cortico-subthalamic coupling become stronger during ipsilateral responses when movement was inhibited, suggesting that coupling may play a functional role in response inhibition. Understanding how motor function is disordered in PD, and investigating the neural substrates underlying this pathology is critical to improving the treatment of this disease. These findings demonstrate the functional significance of the coupling between sparse (30%) networks of neurons in both STN and sensorimotor cortex.

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Poster

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Topic: D.15. Basal Ganglia

Title: How do the basal ganglia control reaching movements?

Authors: J. C. HOUK, *B. W. PETERSON;
Dept Physiol, Northwestern Univ. Med. Sch., Chicago, IL

Abstract: Basal ganglia (BG) output neurons (BGO - found in SNr and GPi) tightly control saccadic eye movements via direct disinhibition of superior colliculus. In somatic reaching, BGOs disinhibit thalamic input to motor cortex (M1) rather than M1 itself, raising questions about efficacy. During well-practiced reaching movements, primate BGOs respond only after the movement has been initiated (Turner & Desmurget 2010). As rodents learn a head-reaching task, most striatal neurons gradually cease firing until only 11% remain active (Tang et al 2007). These findings suggest that BG initiate unfamiliar reaching movements and then drive plastic changes that enable motor cortex to initiate reaching on its own (Exportation). In support, Roy et al (SfN 2008; 378.9) observed that while GPi neurons were only weakly modulated during primary movements monkeys made to a target, less predictable corrective movements were accompanied by large changes at latencies appropriate to trigger the correction. Confirmation of this exportation hypothesis was assembled in Houk et al (2007). We conclude that BG control reaching movements by learning appropriate commands and exporting them to M1 for quicker execution.

Disclosures: J.C. Houk: None. **B.W. Peterson:** None.

Poster

708. Basal Ganglia Anatomy and Physiology

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Topic: D.15. Basal Ganglia

Support: NS045962

Title: Glutamatergic transmission blockade in the striatum reduces dyskinesias in Parkinsonian monkeys

Authors: *S. M. PAPA[1], A. SINGH[2];
[1]Neurol., Emory Univ., Atlanta, GA; [2]Yerkes Natl. Primate Res. Center, Emory Univ. Atlanta, Atlanta, GA

Abstract: Striatal glutamatergic hyperactivity is thought to play a crucial role in the mechanisms of levodopa-induced dyskinesia (LID) in Parkinson's disease (PD). Striatal projection neurons (medium spiny neurons, SPNs) are markedly hyperactive and often exhibit inverted

(bidirectional) firing frequency changes following dopaminergic stimulation. These abnormal physiological responses that are associated with LID expression can be reversed to stable, unidirectional responses by striatal micro-infusion of NMDA receptor antagonist. In addition, the systemic administration (s.c.) of NMDA antagonists reduces LID. However, the impact of localized striatal glutamatergic blockade on dyskinetic behavior has not been assessed. To this end, the NMDA receptor antagonist LY235959 (9 mM; total volume, 10µl) or vehicle (aCSF) was infused into the striatum in one side (0.33 µl/min) of the brain of awake advanced parkinsonian macaques. Striatal injection sites were determined by electrophysiological mapping targeting the posterolateral region of the putamen (the sensory-motor territory). The striatal infusion was followed by a systemic injection of levodopa at predetermined dyskinesigenic doses. Animals were then assessed for motor disability and LID severity using standardized rating scales for primates. Striatal LY235959 significantly reduced LID (total and peak dyskinesia scores) on the contralateral side without compromising the antiparkinsonian action (motor disability scores) of levodopa. The striatal infusion of vehicle did not change LID scores. These findings confirm that glutamatergic transmission in the dysfunctional striatum underlies the release of involuntary movements in advanced parkinsonism.

Disclosures: **S.M. Papa:** None. **A. Singh:** None.

Poster

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Topic: D.15. Basal Ganglia

Support: NS045962

Title: Striatal oscillations in advanced Parkinsonian monkeys and their regulation by glutamatergic transmission

Authors: *A. SINGH[1], S. PAPA[2];
[1]Yerkes Natl. Primate Res. Center, Emory University,, Atlanta, GA; [2]Yerkes Natl. Primate Res. Center, Emory Univ. Atlanta, Atlanta, GA

Abstract: Dopamine depletion in Parkinson's disease (PD) has been associated with abnormal oscillatory activities in the cortico-basal ganglia network (i.e. cortex, globus pallidus, and subthalamic nucleus). However, oscillatory activity in the striatum following dopamine loss and chronic replacement therapy remains poorly defined. Here, we studied striatal oscillations during

"off", "on" and dyskinesia (LID) states in advanced parkinsonian primates. Additionally, glutamate receptor antagonists known to stabilize neuronal firing changes after L-dopa administration were used to regulate the abnormalities in striatal activity. Striatal local field potentials (LFPs) were recorded in MPTP-treated monkeys with standard techniques. The NMDA receptor antagonist LY235959 was injected into the striatum at the recording site before the systemic injection of levodopa. Striatal LFPs were analyzed during "off", "on" (after local artificial CSF or NMDA antagonist and systemic levodopa administration), and subsequently during "on"-with-dyskinesia state. A peak with the higher amplitude (relative power) in the 8-13 Hz (alpha frequency band) was recorded in the "off" state. This peak significantly decreased in the "on" state (local aCSF). However, a peak with higher amplitude in the 13-20 Hz (low-beta frequency band) was observed during "on" state. The NMDA antagonist reduced the peak amplitude in 13-20 Hz during the "on" state. No clear peak was observed in relation to LID. Therefore, reduced peak in 8-13 Hz and increased peak in 13-20 Hz could be associated with the dopamine response in chronically treated severe parkinsonian animals. These results also indicate that the reduction of glutamatergic transmission regulates abnormal striatal oscillations in response to levodopa in the advanced stage of PD.

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Poster

708. Basal Ganglia Anatomy and Physiology

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Topic: D.15. Basal Ganglia

Title: How the cerebellum and the basal ganglia cooperate together for sensorimotor learning ?

Authors: *L. PIDOUX[1,2], A. LEBLOIS[2];
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Abstract: Human speech is a complex sensorimotor skill and vocal learning is one of the most striking cognitive abilities of the brain. As many other complex motor skills, vocal learning involves the basal ganglia (BG)-thalamo-cortical network and the cerebello-thalamo-cortical network in humans. While the BG and cerebellar sub-cortical loops have been shown to interact at least through two different pathways in mammals, the role of their interaction during sensorimotor learning, and in particular during vocal learning, remains undetermined. Songbirds are one of the few accessible animal models for vocal learning, as they have a specialized portion

of their BG-thalamo-cortical circuitry dedicated to song learning. Additionally, a cerebellar projection to the thalamic region adjacent to the song-related thalamic nucleus receiving BG input suggests that BG and the cerebellum may interact during song learning. However, very little is known about song-related circuits in the cerebellum, or about a putative cerebellar function in song learning. We are studying the interactions between BG and cerebellar sub-cortico-cortical loops involved in avian song learning. In order to determine to what extent the cerebellum is involved in song learning and dissect the cerebellar circuits interacting with thalamic and cortical song-related nuclei, we performed two sets of experiments. On one hand, we investigated the physiological mechanisms underlying the integration and transfer of cerebellar signals in the BG using electrophysiological recordings of evoked BG activity following electrical stimulation in the cerebellum. Stimulation of the deep cerebellar nuclei evoked fast excitatory responses in BG neurons located in the song-related BG nucleus Area X. With pharmacological experiments, we found that these responses are mediated by the thalamus before going to the BG and then transmitted to the cortical nuclei LMAN. Our results also suggest that another pathway, linking the cerebellum to the song system by MMAN and HVC, could exist in songbird. On the other hand, we performed cerebellar lesion on adults and juveniles zebra finches (at 30 and 60 dph) to quantify the impact of the cerebellum during song production and learning respectively. We found no or minor differences in adults song production features (duration, pitch) before and after cerebellar lesion. However, syllable duration, pitch and similarity to the tutor are impaired in juveniles.

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Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Stipend

Title: Understanding the role of glutamate in deep brain stimulation of the subthalamic nucleus using enzyme-based microelectrode amperometry in a transgenic mouse model

Authors: *E. S. ARVIDSSON, Å. KONRADSSON-GEUKEN, N. SCHWEIZER, S. PUPE, M. PAPATHANOU, Å. WALLÉN-MACKENZIE;
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Abstract: The underlying mechanisms of the clinical efficacy as well as the side effects of deep brain-stimulation (DBS) of the basal ganglia, more specifically, the stimulation of the subthalamic nucleus (STN), in patients suffering from severe Parkinson's disease, are still unknown. Despite both clinical and pre-clinical characterizations of the anatomical and electrophysiological properties of neurons within this structure, the identification of promoter-specific subgroups of neurons within this area itself has been somewhat neglected. As the STN is considered to be a homogenous structure, highly glutamatergic, we have characterized the expression of Vesicular Glutamate Transporters (VGLUTs) in this area of the mouse brain, to further increase the current understanding of the glutamatergic nature of the STN. In line with previous studies, we have confirmed VGLUT2 to be the predominant subtype. More notably, we have been able to identify a subpopulation within the STN, which is characterized by co-expression of VGLUT2 and the paired-like homeodomain transcription factor 2 (Pitx2). Based on this knowledge, we were able to create a conditional knockout (cKO) mouse line lacking expression of VGLUT2 in this STN subpopulation. These cKO mice displayed hyperlocomotion and decreased latency in the initiation of movement, while preserving normal gait and balance (*Schweizer, Pupe et al, PNAS, May, 2014*). No major cognitive deficits were observed. To investigate the importance of glutamate dynamics in target areas of the STN, the Entopeduncular nucleus (EP) and the substantia nigra pars reticulata (SNr), we are currently implementing enzyme-based microelectrode amperometry (MEA) with a second-by-second time resolution. Our preliminary results suggest that the cKO mice display a reduced glutamate release to the EP. These data correspond to the traditional view of the basal ganglia, where reduced glutamate release onto the EP and SNr will decrease the indirect pathway gated by the STN and hence, increase the action of the direct pathway, and could thus provide the explanation for the observed hyperlocomotion. By utilizing MEA in our cKO mice, we hope to be able to explain the importance of glutamate in the STN network involved in DBS and, hereby, suggest clinical improvement of this therapy.

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Poster

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Title: After effects of microstimulation in human globus pallidus

Authors: *F. LUO, D. CLARK, L. KIM, M. NOOR, Z. KISS;
Dept. of Clin. Neuroscience, Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: Deep brain stimulation (DBS) of the internal segment of the globus pallidus (GPi) is a highly effective therapy for movement disorders such as Parkinson disease and dystonia, yet how it does so remains controversial. The mechanisms of action are likely complex and may be different for different movement disorders. We previously reported cholinergic involvement in simulated DBS (sDBS) applied to entopeduncular nucleus (EP, rodent equivalent of GPi) neurons in brain slice. sDBS applied inside EP induced a prolonged afterdepolarization with increased spiking that was dependent on activation of cholinergic inputs and muscarinic receptors. However, evidence from humans is lacking; mainly inhibition, related to GABA release, has been identified in human GPi in response to microstimulation. Therefore, in order to validate our finding in EP, we sought evidence for increased spiking in patients undergoing dual-microelectrode (distance between 200 and 300 μm) mapping of the GPi. Preliminary data suggest that 10 seconds of high frequency microstimulation (similar to sDBS in brain slice) applied either from the recording electrode or adjacent non-recording electrode could increase post-stimulation spiking in 4 of 9 GPi neurons studied (3 patients). The post-stimulation spiking patterns of these GPi neurons were similar to those of EP neurons observed in brain slice (as well as those described in MPTP treated monkeys). This study provides new insights into the underlying mechanisms of clinical DBS in human.

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Poster

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Title: Acetylcholine evokes spontaneous muscarinic IPSCs in medium spiny neurons overexpressing GIRK channels

Authors: A. MAMALIGAS[1], *C. FORD[2];
[1]Neurosciences, [2]Physiol. and Biophysics, Case Western Reserve Univ., Cleveland, OH

Abstract: Cholinergic interneurons (ChIs) provide the primary source of acetylcholine for the striatum. Firing of striatal ChIs is relevant in various movement and motivated behaviors. Previous studies have shown that ChI active zones do not directly appose MSN dendrites. Nonetheless, it is known that medium spiny neuron (MSN) excitability is modulated by activation of muscarinic receptors (mAChRs). However, the time course over which ChIs signal to MSNs is unclear because acetylcholine does not directly activate any endogenous ion channels on these neurons. Here, a G-protein coupled inwardly rectifying K+ channel (GIRK2) was virally overexpressed in striatal MSNs. The resulting K+ conductance was used to readout the activation of mAChRs using whole-cell voltage clamp recordings. Upon electrical stimulation of ChIs, robust M4-mAChR mediated IPSCs were evoked in GIRK2 expressing MSNs. The kinetics of the evoked M4-IPSCs were rapid relative to other metabotropic IPSCs that have been observed, with a 10-90% rise time of 72 +/- 3ms and a 20% width of 484 +/- 50ms. This suggests that a high concentration of acetylcholine likely activates mAChRs on MSNs. IPSCs evoked at minimal stimulation intensities exhibited successes and failures, suggesting that stimulation of a single ChI can elicit an IPSC. Subsequently, paired recordings of ChIs and MSNs were performed to determine if an action potential elicited in a ChI could evoke an M4-IPSC in a postsynaptic MSN. When an action potential occurred in a ChI, an IPSC was reliably observed in the synaptically paired MSN, indicating that mAChRs on MSNs reliably encode acetylcholine release resulting from the action potential of a single ChI. Finally, spontaneous M4-IPSCs, eliminated by TTX, were also observed in GIRK-expressing MSNs, suggesting that the 2-5 Hz tonic firing pattern of ChIs can be encoded by mAChRs. Thus despite the tonic background firing of cholinergic interneurons, the results suggest that release of acetylcholine may produce a local and phasic activation of muscarinic receptors in MSNs.

Disclosures: A. Mamaligas: None. **C. Ford:** None.

Poster

708. Basal Ganglia Anatomy and Physiology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.15. Basal Ganglia

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CIHR Doctoral Fellowship

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Title: Enkephalin disinhibits striatal patches via the delta opioid receptor

Authors: *S. NEUFELD[1], M. R. BANGHART[1], N. MULDER[1], B. SABATINIT[1,2];
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Abstract: Opioid peptides and their receptors are prominent in the dorsal striatum, a brain region critical for the generation of purposeful movements and goal-directed behavior. Mu-opioid receptors (MORs) are highly enriched in patch compartments (or striosomes), distinct limbic microcircuits embedded within the predominately sensorimotor dorsal striatum (known as the matrix). There is growing evidence that imbalances of patch-matrix activity in the striatum correlate with Huntington's disease, Parkinson's disease, and drug addiction. Previous work has revealed patch specific suppression of inhibition by the MOR agonist DAMGO. It is not known which populations of pre- and post-synaptic neurons within patches are targeted by DAMGO, and whether action of endogenous opioid peptides, which can activate multiple opioid receptors, shifts the balance of patch-matrix activity. Using a combination of transgenic mice, optogenetics, and pharmacology we identified the inhibitory synapses suppressed by opioid peptides, investigated the underlying receptors, and assayed the overall effect of opioid signaling on patch and matrix output. We validated a transgenic mouse line that allows simultaneous observation of patches and post-synaptic cell identity. Using these mice we confirmed that enkephalin (enk), the only endogenous ligand for MORs in striatum, suppresses local inhibition onto both direct and indirect pathway striatal projection neurons selectively in patches. By virally introducing cre-dependent ChR2 into mice expressing cre-recombinase under cell-type specific promoters, we identified local collaterals of indirect pathway striatal projections neurons as the major pre-synaptic target of enk. To our surprise, selective pharmacology revealed that delta-opioid receptors, not MORs, dominate the suppression of inhibition. Finally, we assayed the overall

effect of enk on the output of patch and matrix neurons by stimulating corticostriatal input. We found that enk increased spike frequency in the patches, but not in the matrix. Our data indicate that enk release could shift the balance of patch-matrix signaling by selectively disinhibiting patch neurons via delta-opioid receptors.

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Poster

708. Basal Ganglia Anatomy and Physiology

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Title: VTA-AcbSh circuitry analysis and modulation of the response to novelty

Authors: ***H. LI**, M. CRANSTON, C. MACTUTUS, M. AKSENOVA, B. KANTOR, R. BOOZE;
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Abstract: The mechanism of how stimulation of specific pathways modulates behaviors, such as the response to novelty, is still unknown; however, previous studies show that dopamine is critical for orientation and response to novel stimuli. To investigate the role of particular pathways in responses to novelty, pharmocogenetic techniques (retroDREADDs) were used to modulate the G protein-coupled receptor (GPCR) system during a novel experience. First, the selectivity and expression of the AAV-hM3D DREADD was confirmed in primary neuronal cell cultures. Second, we selectively expressed AAV-CAG-CRE-GFP in nucleus accumbens shell (AcbSh) and infused AAV-hSyn-DIO-hM3D (Gq)-mCherry (a presynaptic enhancer in the presence of its cognate ligand clozapine-N-oxide, CNO) in the posterior ventral tegmental area (VTA). We found AcbSh CRE-GFP was retrogradely transported to the pVTA, which then triggered hM3D-mCherry production in neurons of the VTA which had connectivity with the AchSb. Finally, activation of the DREADD *in vivo* by administration of CNO altered the

response to novelty (removal of background white noise) in an open-field task ($p \leq 0.05$), without altering locomotor activity levels. Immunohistological analysis confirmed production of hM3D in neurons of the posterior limb of the VTA; these AcbSh projection neurons were both dopaminergic and non-dopaminergic. Collectively, stimulation of the circuit connections from the pVTA to the AcbSh altered selective responses to novelty, without altering gross locomotor behavior. In sum, selective retroDREADD pathway analysis provides insights into the circuitry underlying particular behaviors (e.g., novelty) and provides a unique therapeutic approach.

Disclosures: H. Li: None. **M. Cranston:** None. **C. Mactutus:** None. **M. Aksenova:** None. **B. Kantor:** None. **R. Booze:** None.

Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

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Program#/Poster#: 709.01/U24

Topic: D.17. Voluntary Movements

Support: Aix-Marseille University foundation A*Midex; Coord-Age project

Title: Task properties determine age-related differences in force control

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Abstract: Accuracy and stability of force control results from the temporary coalition of multiple subsystems (e.g. cognitive and muscular systems), as well as from central integration of different sensory feedback loops, which finally depends on task constraints. Age-related differences in rapid force production (RFP) and isometric force maintenance (IFM) tasks have been well described in the literature (Christou & Carlton, 2001; see Morrison & Newell, 2012 for an overview). However, an experimental paradigm is still lacking to investigate the combination of these processes. The present study addressed this issue by comparing how age-related changes manifest when RFP and IFM are performed in separation or combined in a Fitts-like paradigm. We expected age-related differences to be differently expressed in the combined than in the separated tasks. Young and old adults performed RFP and IFM tasks at various force levels (10, 20, 40, 60, 80% of MVC). Both tasks were combined by a Fitts-like paradigm in which both distance (D) and target width (W) were manipulated (ID 1, 2, 3, 4). For RFP the time to peak (equivalent to MT) was detected and Brinley plot slopes were analyzed. SD and multi-scale-

entropy area (MSE) were calculated for IFM. Brinley plots showed multiplicative slowing in all task conditions, with higher slopes for the separated (4.33) than the combined tasks (W: 1.40; D: 2.20). In the separated IFM task, no age-related differences were found. In the combined task older showed higher variability, W: $F(1,20) = 5.05$, $p = .04$, $\eta_p^2 = .202$; D: $F(1,20) = 20.76$, $p < .01$, $\eta_p^2 = .509$, and for MSE an age by ID interaction, W: $F(3,20) = 4.26$, $p = .01$, $\eta_p^2 = .176$; D: $F(3,20) = 3.34$, $p < .05$, $\eta_p^2 = .143$, indicating different evolutions of MSE over IDs. As predicted, the comparison of RFP and IFM showed different age effects in separated and combined tasks, respectively. This suggests that the combined task is more than the simple juxtaposition of RFP and IFM. It also indicates that the consequences of reorganizations occurring in the aging force control system are strongly sensitive to task properties. This should be taken into account in future studies References Christou, E. A., & Carlton, L. G. (2001). Old adults exhibit greater motor output variability than young adults only during rapid discrete isometric contractions. *J Gerontol A-Biol, 56*(12), B524-B532. S. Morrison and K. M. Newell. (2012). Aging, Neuromuscular Decline, and the Change in Physiological and Behavioral Complexity of Upper-Limb Movement Dynamics. *J Aging Res, (2012),* 14 pages. doi:10.1155/2012/891218.

Disclosures: S. Vieluf: None. **J. Temprado:** None. **R. Sleimen-Malkoun:** None.

Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 709.02/U25

Topic: D.17. Voluntary Movements

Title: The effects of dual tasking on bimanual grip force control in older adults

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Abstract: Dexterity declines with age, due, in part, to difficulties maintaining submaximal forces (Marmon et al., 2011). While motor unit reorganization is thought to contribute to increased force variability in older adults (Galganski et al., 1993), age-related declines in attentional capacity may also play a role in difficulties maintaining static force (Voelcker-Rehage et al., 2006). Further, most studies have examined fine force control using unimanual tasks despite the need to simultaneously control force production in both hands during many activities of daily living (Kilbreath and Heard, 2005). Thus, the purpose of this study was to examine the

interplay between age, attentional demands, and grip force production under different force conditions. Ten healthy young (mean age 19.0 y) and 10 older (mean age 70.7 y) adults generated visually-guided unilateral (dominant, non-dominant) and bilateral grip forces at 5 or 20% of each individual's maximum voluntary contraction (MVC) using hand-held dynamometry. Tasks varied in difficulty by having participants increase force to either 5 or 20% of MVC from baseline or increase/decrease force from and to a controlled force level - i.e. increasing force from 5-20% or decreasing force from 20-5% of MVC (5% to 20%, 20% to 5%). All tasks were performed with and without a concurrent cognitive load involving counting backwards by seven. Following target acquisition, participants maintained force for 4 sec. Dependent measures included force maintenance variability and force initiation time. When increasing force from baseline, static force variability at 20% MVC did not significantly differ between age groups and was unaffected by hand, hand configuration (i.e. unimanual vs. bimanual), or cognitive load. In contrast, significant age differences in force variability were seen when maintaining very low submaximal forces (0-5% task) but only during the cognitive load condition ($p < .001$). In tasks requiring controlled decreases in force from 20% to 5%, variability did not differ between age groups when the task was performed unimanually and in the absence of any cognitive task. However, bimanual force tasks, coupled with increased attentional demands led to pronounced age-related differences in variability ($p<.001$). Force initiation time was consistently longer in tasks requiring a force increase in both age group ($p<.001$) with cognitive loading leading to longer initiation times in the older compared to the younger group ($p<.001$). These results extend the previous results demonstrating attentional demands in submaximal force control in older adults and underscore the importance assessing bimanual grip force control.

Disclosures: H. Hibino: None. **R. Carlson:** None. **S.H. Brown:** None.

Poster

709. Finger and Grasp: Age, Pathology, and Physiology

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Program#/Poster#: 709.03/U26

Topic: D.17. Voluntary Movements

Title: Sensorimotor memory and sensorimotor integration abilities dissociate in a post MCA stroke population

Authors: *B. KC, K. FERCHO, L. A. BAUGH;
Biomed. sciences, Univ. of South Dakota, Vermillion, SD

Abstract: Current bedside diagnostics used for the assessment of the loss of hand function post-stroke examine limited aspects of motor performance. Further, they are not sensitive to subtle changes that can cause deficits in everyday object manipulation tasks. Efficiently lifting an object entails a prediction of required forces based on intrinsic features of the object (sensorimotor integration), and short-term updates in the forces required to lift objects that are poorly predicted (sensorimotor memory). Therefore, a successful object manipulation is a complex neurological event with multiple cortical areas involved. Unfortunately, this complexity is not represented by the existing assessment tools used in clinics for both diagnostic and rehabilitative purposes. The presented research examined the ability of two object lifting tasks designed to assess sensorimotor memory and sensorimotor integration to classify middle cerebral artery (MCA) stroke deficits relative to age-matched control participants. Age-matched control participants and MCA stroke participants completed two tasks. The first was designed to assess sensorimotor memory integration abilities. Participants were presented with large wood or brass blocks following trials of small and medium sized blocks from the same size-weight families. To accurately predict the weight of the larger cubes, sensorimotor integration of object size and apparent material is required during the first lifts of the large cubes. In the second task, participants were required to lift a series of size-weight blocks of different colors. One color signified an inverse size-weight relationship that required the modification of short-term sensorimotor memory to efficiently lift. In both tasks, lifting forces were examined and compared to age-matched control data. As predicted, individual stroke participants demonstrated dissociable errors in each of the lifting tasks. A small portion of stroke patients performed at control levels in both tasks. However, most stroke participants showed a deficit in sensorimotor memory, sensorimotor integration, or both lifting scenarios as revealed through a detailed analysis of lifting forces. The presented research demonstrates MCA stroke participants may have deficits in one or more components required for the successful manipulation of hand-held objects, and these deficits can be detected in an object lifting task. Further, by identifying specific deficits in lifting ability, targeted rehabilitation regimens may be possible.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

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Program#/Poster#: 709.04/U27

Topic: D.17. Voluntary Movements

Title: The influence of transcranial direct current stimulation timing on motor skill acquisition in older adults

Authors: ***B. J. POSTON**[1], A. JACKSON[1], Z. RILEY[2], E. L. HEISLER[3], R. R. WALSH[3], J. L. ALBERTS[3];
[1]Kinesiology and Nutr. Sci., Univ. of Nevada Las Vegas, Las Vegas, NV; [2]Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN; [3]Cleveland Clin., Cleveland, OH

Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that has been shown to enhance motor performance in older adults. However, the optimal tDCS parameters for improving motor function in older adults have not been established. The purpose was to determine the influence of the timing of tDCS on motor skill acquisition in older adults. The study was a sham-controlled, crossover experimental design. Nine older adults (71 ± 6.9 yrs) participated in 3 experiments that were each separated by a 7 day washout period. Each experiment involved practice of a precision grip task (primary practice task; PPT) performed in association with 1 of 3 tDCS interventions (BEFORE, DURING, and SHAM) in counterbalanced order. The PPT involved matching a target sine wave (target force range: 5-25% of maximum) for 10 trials of 30 seconds each followed by a 90 s rest (total practice or stimulation time = 20 min). Thus, tDCS was applied either before or during motor practice for 20 minutes. tDCS was applied to the scalp area overlying the 1st dorsal interosseus muscle representation area of the primary motor cortex. The tDCS current strength was held constant at 1 mA in both of the treatment conditions. SHAM stimulation was applied in the same manner according to established blinding procedures. The force error (primary outcome measure) during the PPT was quantified as the average error in force relative to the target force. tDCS applied both before and during the PPT lead to better performance (21% and 25% lower force error, respectively) compared to SHAM stimulation. However, there was a non-significant (~4%) difference in force error between the BEFORE and DURING conditions. The findings indicate that a single application of tDCS applied either before or during motor practice can increase the rate of motor skill acquisition in older adults.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.17. Voluntary Movements

Support: NIH R01NS079569

Title: Postnatal motor cortex stroke alters development of the rubrospinal system

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Physiology, Pharmacol. and Neurosci., City Col. of the City Univ. of NY, New York, NY

Abstract: Motor cortex stroke during development is a leading cause of cerebral palsy (CP), a common developmental disorder affecting 2-3/1000 births. In unilateral CP the corticospinal system (CS) from the damaged cortex fails to develop strong contralateral connections with the spinal cord, and the CS from the less affected hemisphere develops bilateral spinal connections and leads to motor map impairments and behavioral deficits. The CS and the rubrospinal system (RS) are the two components of the lateral motor systems for skilled limb control. It is unknown if CS miswiring enhances or corrupts development of the RS. In this study, we addressed two questions: does M1 stroke alter development of the (1) red nucleus (RN) motor map, and (2) the pattern of rubrospinal tract (RST) terminations in the cervical spinal cord. We studied these questions in the cat, a species for which we have detailed information about the timing of development of both the corticospinal tract and RST. Our hypothesis is that the RS will show augmented growth of the RN motor map and RST from the ipsi-lesion side if there is compensation for the injured CS. To model CP, a unilateral stroke (photothrombotic: rose bengal, 20mg/Kg i.v.; 15 min activation with 530 nm LED light source) was induced in the forelimb region of motor cortex at postnatal week 5, which is a key period of CS and RS development in cats. We examined RST terminations labeled in C8-T1 with anterograde tracers (BDA or AF488DA) injected in the RN. In terminal experiments at weeks 7-8, the RN motor maps on the ipsilesional and contralesional sides were examined using microstimulation. We found low variability in stroke lesion size. All animals showed a placing deficit with the contralesional forelimb, consistent with forelimb motor cortex lesion. The RN map on the side in which M1 was ischemic appeared normal. Surprisingly, the map on the contralesional side was abnormally small with elevated thresholds compared with the ipsilesional side. Analyses of RST terminations are ongoing. These are the first results to link postnatal lesion of CS with altered development of RS function. The diminished RS from the contralesional side suggests the intact CS out competes the RS for access to spinal motor circuits. On the basis of changes in the RN motor map, we did not find evidence that the RS spontaneously compensates for the damaged CS during development.

Disclosures: P.T. Williams: None. **J.H. Martin:** None.

Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.17. Voluntary Movements

Support: CR32I3_13826

Title: Neural correlates of passive forefinger kinematics: effects of amplitude, direction and velocity

Authors: *J. DUENAS[1], J. SULZER[2], P. STAEMPFLI[3], M.-C. HEPP-REYMOND[3], S. KOLLIAS[4], E. SEIFRITZ[3], R. GASSERT[1];
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Abstract: While the differential neural responses to passive and active movements have been well-studied, there is little understanding as to the relationship between the degree of passive movement and brain activity. Afferent input includes receptors that are sensitive to both length and rate of length changes in the musculotendon unit. Thus, we hypothesized that these parameters are represented differently in the brain during passive forefinger movement. We measured whole brain blood oxygenation level dependent (BOLD) signal using functional magnetic resonance imaging (fMRI) in response to parametric changes in passively induced forefinger kinematics. Nineteen healthy participants were exposed to combinations of forefinger flexion and extension imposed by a MR-compatible robotic manipulandum, which also measured forefinger interaction forces. Each subject was exposed to passive forefinger movements at three different levels of amplitude and velocity, separately in flexion and extension. We used a parametric analysis and modeled forefinger interaction force as a regressor of no interest to account for variance due to different mechanoreceptor input. We found that contralateral primary and secondary somatosensory regions, as well as putamen and ipsilateral cerebellum were positively linearly correlated with increases in passive movement velocity. Increases in displacement amplitude showed a non-linear decrease in activity within bilateral secondary somatosensory regions, whereas increases in static forefinger position presented an increase in the aforementioned areas. These insights will provide a greater understanding of the neural representation of kinematic variables, which could lead to improve therapeutic strategies for severely impaired patients following brain injury.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

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Topic: D.17. Voluntary Movements

Support: James McDonnell Foundation 90043345

Title: Correlated deficits in finger individuation following unilateral stroke in the affected and unaffected hand

Authors: *N. EJAZ[1], J. XU[2], B. HERTLER[5], M. BRANSCHEIDT[5], M. WIDMER[5], N. KIM[2], M. HARRAN[6], J. C. CORTES[6], A. V. FARIA[3], P. A. CELNIK[4], T. KITAGO[6], A. LUFT[5], J. W. KRAKAUER[2], J. DIEDRICHSEN[1];
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Abstract: Following a stroke, the recovery of dexterous hand function (Xu et al. 2015) largely occurs within the first few months. This recovery is likely as a result of cortical reorganization in the affected hemisphere. However, the extent of reorganization in the unaffected hemisphere in this recovery process is currently unclear. Here we investigated cortical reorganization in both hemispheres by quantifying behavior deficits in both paretic and non-paretic hands following stroke. Patients with a unilateral stroke (N=48) and healthy age-matched controls (N=14) were asked to perform a finger individuation task and their behavioral performance was assessed longitudinally at 5 time points (between weeks 1-52 after stroke onset). Subjects made individuated force presses with an instructed finger at four different force levels (between 20-80% of maximum voluntary contraction), and the patterns of involuntary forces produced by the passive fingers of the instructed hand (enslaving), and by all fingers of the uninstructed hand (mirroring) were recorded. These enslaved movements are proposed to have - in part - a cortical origin (Zatsiorsky et al. 2000), and provide a window into cortical reorganization during recovery. We found increased enslaving of the fingers of the affected hand as well as increased mirrored movements in the unaffected hand when the affected hand moved. Both enslaving and mirrored movements reduced during recovery at roughly the same time-course. Apart from the overall enslaving, we also studied patterns of enslaving themselves - i.e. enslaving between pairs of fingers - after normalizing for severity. Stroke caused this pattern to become abnormal in comparison to the enslaving patterns for healthy controls. Strikingly, during weeks 1-4, the enslaving patterns in both affected and unaffected hands were equally deviated from the healthy enslaving pattern, with no significant difference between hands. Furthermore, during recovery

the enslaving patterns for both hands started to increasingly resemble the healthy enslaving patterns. This result suggests a shared bilateral representation for both hands across hemispheres that is disrupted through stroke, and subsequently recovers. Consistent with this idea, the enslaving and mirrored movement patterns were more correlated across the affected and unaffected hands within the same patient, than across hands across individuals. Together, these results argue that skilled finger movement representations are co-operatively organized across both hemispheres. Damage to even a single hemisphere potentially results in bi-hemispheric cortical reorganization to recover hand function over time.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

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Intramural Research Grant 27-4 and 27-8 for Neurological and Psychiatric Disorders of National Center of Neurology and Psychiatry, Japan

Title: White matter changes in pianists with focal hand dystonia

Authors: *K. KITA[1], K. UEHARA[2], S. FURUYA[3], R. OSU[4], T. SAKAMOTO[5], T. HANAKAWA[2];
[1]Chiba Univ., Chiba, Japan; [2]Dept. of Advanced Neuroimaging, Integrative Brain Imaging Center, Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [3]Sophia Univ., Tokyo, Japan; [4]ATR, Kyoto, Japan; [5]Natl. Ctr. of Neurol. and Psychiatry Hosp., Tokyo, Japan

Abstract: Musician's dystonia (MD) is a task-specific movement disorder characterized by abnormal posturing and loss of fine motor control. Typically in MD at the hand, the affected fingers become uncontrollable mostly during playing a specific musical instrument, and respond normally to other motor activities. For pianists, MD can be highly disabling and terminate their professional musical careers. Although the neurological origins of this disorder are not yet completely clarified, it is probable due to dysfunctional or maladaptive brain plasticity. For instance, gray matter reorganization in basal ganglia and abnormal brain activities in motor and premotor area were reported in previous studies. Recently, we also have revealed abnormal cerebellar activation of pianists with MD during continues finger-tapping task. In this study we focus on structural, especially white matter, abnormalities underlying pathophysiology of MD. The diffusion tensor imaging (DTI) was acquired from 14 experienced healthy pianists (EP), 14 non-experienced pianists (NP) and 18 pianists with MD by using 3T MRI scanner. All pianists with MD had dystonic symptom at either the right hand or both hands. EP are pianists who have majored the keyboard, whereas NP are healthy pianists who enjoy playing piano as a recreation. Functional anisotropy (FA) is one of the most popular parameter computed from DTI. FA represents size and density of axons and degrees of myelination and shows structural organization of white matter fibers. We performed a voxelwise statistical analysis of FA values using tract-based spatial statistics (TBSS, Oxford Centre for Functional MRI of the Brain's software library (FSL5.0.8)). A comparison of FA values across the groups found significantly greater FA values in corpus callosum, corticospinal tract, posterior and anterior thalamic radiation in EP compared to pianists with MD (the statistical threshold was set at $p < 0.05$, FWE corrected). Although the difference of FA values between NP and pianists with MD was not significant, it showed the same tendency with the comparison between EP and pianist with MD: FA values in the aforementioned areas tend to be greater in NP compared to pianists with MD. The present study suggests that abnormal structural organization in the white matter in cortical and sub cortical areas may be related to pathophysiology of MD.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

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Topic: D.17. Voluntary Movements

Support: NIH Grant NS046367

Title: Effects of age and handedness effects on hand motor recovery after sensorimotor cortex lesions in Macaca mulatta

Authors: *W. G. DARLING[1], S. M. HYNES[1], M. A. PIZZIMENTI[2], D. L. ROTELLA[1], J. GE[3], K. S. MORECRAFT[3], R. J. MORECRAFT[3];
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Abstract: Recovery of hand/digit function after unilateral damage to sensorimotor areas of the brain may be affected by age if potential for neuroplasticity decreases with increasing age. Strength of handedness may also affect recovery of hand function, especially after damage to the more dominant hemisphere, because reduced interhemispheric inhibition (IHI) to the non-lesioned hemisphere may improve skillful use of the ipsilesional hand and promote learned nonuse of the contralesional hand. Larger volume lesions to a strongly dominant hemisphere would be expected to decrease IHI to a greater extent and perhaps contribute to even poorer recovery of contralesional hand function. We examined effects of age and strength of hand preference on recovery of hand motor function in 18 adult rhesus monkeys with a wide range of: surgically placed lesion volumes to sensorimotor areas of the brain (112 - 1001 mm3), ages (3.8 - 20.7 years) and strengths of hand preference (1.1 - 96) as measured by handedness index (Nudo et al. 1992, J Neurosci 12:2918). Multiple linear regression was used to assess the effects of these variables on recovery of skill in reaching and manipulation. Performance of reaching and manipulation were quantified in modified dexterity board and modified movement assessment panel tasks as described previously (Darling et al. 2009, Exp Neurol 220:90). Overall, total white matter lesion volume was the strongest predictor of the deficits in upper limb motor function and recovery of reaching and fine hand/digit motor function. Gray matter lesion volume, age and strength of hand preference generally made rather small, if any, contribution to the prediction of post-lesion deficits and recovery of upper limb function. Also, effects of age and lesion volume on neuroplastic responses in the corticospinal projection (CSP) to C5-T1 from ipsilesional supplementary motor cortex (iM2) were studied in 8 monkeys (McNeal et al. 2010 J Comp Neurol 518:226; Morecraft et al., 2015 J Comp Neurol 523:669). Younger animals exhibited larger increases in estimated numbers of boutons in spinal laminae of C5-T1 relative to controls than older subjects after M1+LPMC lesions, suggesting age-related effects. In contrast, after M1+LPMC+anterior parietal lobe lesions there was a reduction in estimated number of iM2 CSP boutons relative to controls that depended primarily on lesion volume and was relatively unaffected by age. Overall, these data suggest that white matter lesion volume primarily affects hand motor recovery with little effect of age and handedness whereas age affects the M2 CSP neuroplastic response following frontal lobe lesion but not after frontoparietal injury.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 709.10/U33

Topic: D.17. Voluntary Movements

Title: The effect of developmental dyslexia on grip and load force coordination while holding an object during cyclical arm movement

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Abstract: Developmental dyslexia is a common neurobiological syndrome that affects individuals' reading and writing ability but has no effect on their intelligence. Dyslexia is also associated with individuals' poor performance in sensorimotor tasks, such as postural control and gait. Recently, we found that dyslexic children have deficits in visuomotor processing during a visually guided isometric manipulation task (i.e., ramp up and hold), but have no changes in grip (GF) and load force (LF) coordination. However, the manipulation task performed could be considered very simple and could have concealed possible alteration in GF-LF coordination in this population. Hence, we compared indices of GF-LF coordination of dyslexic and normal reader children during vertical, continuous oscillation of a free-moving object. Nine dyslexic and 9 control children between 9 and 12 years-old were asked to hold an instrumented object using a tripod grasp and to move it continuously up and downward, with peak to peak amplitude of 20 cm, in two frequencies, 0.8 and 1.2 Hz, during 12 seconds, repeating it three times for each frequency. The GF scaling was assessed by GF to LF ratio (GF/LF), GF modulation was evaluated by GF gain and GF offset (respective slope and y-intercept of the linear best fit of GF-LF diagrams), and GF-LF directional and temporal coupling were estimated by the maximum coefficient of a cross-correlation function (rmax) and by its respective time-lag. For GF/LF and GF offset, results revealed no effect of group and no group*frequency interaction, but revealed higher GF/LF and GF offset at 1.2 than at 0.8 Hz. Time-lag was around zero and neither main effect of group and frequency nor group*frequency interaction were observed. Regarding rmax and GF gain results revealed that dyslexic presented lower rmax and GF gain than non-dyslexic children, mainly at 1.2 Hz oscillation task and that rmax was higher in 1.2 than at 0.8 Hz. These results indicate that dyslexic children are as good as non-dyslexic ones in scaling GF with the changes in LF. Also, dyslexics are able to predict the consequences of their arm motion in the digits-object interaction, adjusting GF with the changes in LF with virtually no delay. However,

the directional coupling between GF and LF is altered (lower directional coupling) and GF modulation is negatively affected in dyslexic children. These findings corroborate the idea that dyslexic children present deficits in sensorimotor coordination and that such deficits could be associated with structural changes in specific brain areas as the posterior parietal cortex and portions of the cerebellum which have been linked to GF-LF coordination.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 709.11/U34

Topic: D.17. Voluntary Movements

Support: James McDonnell Foundation 90043345

Title: Changes in neural activity patterns during recovery of fine finger control after stroke

Authors: *J. XU[1], N. EJAZ[4], B. HERTLER[5], M. BRANSCHEIDT[5], M. WIDMER[5], N. KIM[1], M. HARRAN[6], J. C. CORTES[6], A. V. FARIA[2], P. A. CELNIK[3], T. KITAGO[6], A. LUFT[5], J. W. KRAKAUER[1], J. DIEDRICHSEN[4];
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Abstract: Fine finger control recovers maximally during the first months after a stroke, and is largely independent from recovery of strength (Xu et al. 2015, NCM). The neuronal changes that underlie this recovery in the human brain are largely unknown. Using fMRI, a number of previous studies have documented changes in the overall BOLD signal during hand movements. Recent studies from our lab indicate that the structure of digit representations in sensorimotor cortex, in the form of digit-specific pattern distances, is well-organized and consistent across time and individuals. Changes in these fine-grained patterns of neural activity are a more sensitive assay of neural plasticity. Patients with acute onset unilateral cortical or subcortical strokes and a moderate initial impairment in hand function (N=37) and age matched controls (N=11) were tested longitudinally at 5 different time points over one year (week 1, 4, 12, 24, and 52). In the scanner they produced individuated isometric force presses with one of 4 fingers of either the affected or the unaffected hand. Multivariate pattern analysis was used to analyze

fMRI activity in motor and premotor cortices, which was decomposed into a) overall BOLD activity for each hand, b) strength of digit representation, i.e. overall mean distance between finger-specific activity patterns, and c) digit representation structure, i.e. pattern of distances for all digit pairs. Our results showed a reduction of overall average activation in ipsilesional M1 and PMd initially after stroke, which normalized during recovery. The corresponding digit patterns were also disrupted, and recovered in the first 12 weeks, though never reached the level of healthy controls. Digit pattern strength was strongly correlated with a behavioral measure of individuation in the paretic hand, suggesting recovery-related structural change in the peri-infart and premotor areas in the lesioned hemisphere. Furthermore, compared to healthy controls, the overall digit representation structure in both lesioned and non-lesioned hemispheres, when either the paretic or non-paretic hand moved, also showed an initial disruption and subsequent recovery, approaching normal structure with a similar temporal course of behavioral recovery. This result coincides with recovery pattern of enslaving profiles in both paretic and non-paretic hands, suggesting a bi-lateral involvement in the recovery process. In summary, our results indicate that recovery of the ability to individuate fingers is associated with plastic changes in motor and premotor areas of the lesioned hemisphere, and a possible functional role of the contra-lesional hemisphere.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 709.12/U35

Topic: D.17. Voluntary Movements

Title: Abnormal eeg oscillations in writer's cramp

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Abstract: Writer's cramp (WC) is a type of focal dystonia involving the sensorimotor circuit, probably with a heterogeneous biological, environmental and psychological background. Many

kinds of intervention have been already attempted, with limited effectiveness and beneficial duration for the patients. EEG based Brain Computer Interface (BCI) has been recently shown to be a promising tool for the rehabilitation from this disease. Nevertheless, no exhaustive EEG study is yet available to determine which feature should drive the neurofeedback to achieve the most effective results. Therefore, the aim of the present study was to identify the most reliable EEG biomarker of WC. The EEG responses of seven WC patients (age 50±15, 4 males and 3 females, onset age 45±15) were examined and their neurophysiological behaviour was investigated during two different tasks: (1) the execution of a pinch grip movement performed with the right affected hand (ME) and (2) its kinesthetic imagination (MI). Time-frequency analysis over the 5-45 Hz frequency band was performed in terms of event-related spectral perturbation (ERSP) followed by a bootstrap analysis with significance at 95%. From the well-known literature on healthy subjects, a significant power decrease, i.e. negative ERSP, during both the tasks was expected in the μ band (around 10 Hz) and the β band (around 20 Hz) over the sensorimotor cortices, especially the contralateral, compared to a baseline period selected during the rest before the task. Moreover, a significant power increase, i.e. positive ERSP, in the β band was expected within one second from the termination of the task. Nevertheless, preliminary results in WC patients showed a significantly abnormal power increase in the high β band (above 25 Hz) during the ME (five patients) and the MI (seven patients) tasks, in line with the limited previous literature on EEG in WC. Additionally, a clear suppression of the negative ERSP in the μ band was seen during the ME and the MI tasks in six patients. Furthermore, an unexpected and significant power decrease in the μ as well as the low β band was observed at the end of the task period in five out of seven patients. Preliminary results showed that, besides the abnormality in the high β band previously suggested in the literature, other abnormal patterns - still far from the healthy case - could be identified in WC patients. Furthermore, the investigation on the relationship between the different frequency components and their behaviour during the occurrence of the dystonic symptoms could provide a support for these first interesting outcomes and clarify whether pathological or compensatory mechanisms led to the observed abnormal EEG patterns.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

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Topic: D.17. Voluntary Movements

Support: Grant-in-Aid for Scientific Research 15H05358

Title: Abnormalities of the finger movements in musician's dystonia

Authors: *S. FURUYA[1], K. TOMINAGA[2], F. MIYAZAKI[2], E. ALTENMÜLLER[3];
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Abstract: Musician's dystonia (MD) manifests itself through repetitive and precise use of specific body portions, and causes involuntary movements and muscular cramping. Compared with other forms of dystonia such as writer's cramp and cervical dystonia, little has been addressed about neuronal and computational mechanisms underlying production of dystonic movements in MD. MD has therefore a high risk of misdiagnosis, possibly which occasionally causes injection of Botulinum Toxin into an intact finger. The present study addressed effects of MD on hand kinematics during musical performance. Using a dataglove, we recorded dynamic modulation of hand posture while healthy pianists and pianists with FD were playing the piano. The results demonstrated that the patients displayed limited finger extension and intact finger flexion compared with the healthy controls. Principal component (PC) analysis determined three patterns of fundamental joint coordination for both of the two groups. The overall patterns of the first two PCs described less individuated movements between the affected finger and a finger responsible for striking a key for the patients compared with the controls. The third PC that consisted of the individuated movements between the middle and ring fingers was evident during a sequence of strikes with these fingers for the healthy controls, which was however absent in the patients. Accordingly, the patients showed more pronounced rhythmic variability of keystrokes during this sequence of strikes than the controls. A stepwise multiple regression analysis identified less accurate keystrokes for individuals who moved the affected and striking fingers in a less individuated manner. Taken together, the current findings suggest that MD reorganizes joint coordination patterns so as to degrade independent control of movements across fingers and thereby impairs motor precision.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

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Program#/Poster#: 709.14/U37

Topic: D.17. Voluntary Movements

Title: Neural correlates of error processing in the elderly

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Abstract: With increasing age, cognitive control processes steadily decline. Prior research indicates that healthy elderly have a generally intact performance monitoring system, i.e., they perform accurately on tasks assessing motor inhibition, but show specific deficits in error awareness, i.e., the ability to detect committed errors. The electrophysiological correlates of the elderly's deficits in error processing and detection to date remain elusive. Here, we examine the neural processing of errors in healthy elderlies by analysing the error (-related) negativity (Ne/ERN) and the error positivity (Pe) using an adapted version of the Go/Nogo task. For detected errors, 20 elderly participants (mean age 57.0 ± 8.1 years) showed a significantly more negative peak amplitude of the Ne/ERN and a significantly more positive Pe peak amplitude when compared to correct responses. A subgroup analysis of those participants who committed a sufficient number of undetected errors revealed that compared to correct responses the Pe amplitude was similar, while the Ne/ERN peak amplitude was more negative for undetected errors. Data suggest that the neurophysiological patterns of error processing and error detection previously reported in young participants can also be observed in the elderly. Thus, the known electrophysiological correlates of committed errors are stable, irrespective of age. In addition, a sub-clinically depressed mood seems to have a detrimental impact on error detection performance and performance evaluation by depleting cognitive resources which are necessary for a good task performance.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 709.15/U38

Topic: D.17. Voluntary Movements

Title: Effects of tactile feedback on manual function in patients with type II diabetes

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Abstract: Control of manual function requires the optimal integration of sensory and motor systems. Pathology to either of these systems leads to suboptimal dexterity and manual object manipulation. Changes to sensory function is well documented in individuals with Type II Diabetes (T2D); however, the impact of the disease on motor function of the upper extremity has not been thoroughly investigated. The purpose of this study was to evaluate the effects of altered tactile feedback on manual function in patients with T2D (n = 10; 7 females, 3 males) compared to age- and sex- match control subjects (n = 10; 7 females, 3 males) (age = 58 ± 6 years).Tactile sensation, proprioception, and motor function of the dominant hand was assessed in both groups in three conditions: at baseline, after a 1mL injection of 2% lidocaine administered at the carpal tunnel (median nerve block at the wrist, WB), and after a 1mL injection of 2% lidocaine administered medially to the brachial artery at the antecubital fossa (median nerve block at the elbow, EB). The injections used allowed for well-controlled, safe, and effective temporary suspension of tactile sensation distal to the wrist and elbow. We hypothesized that if tactile sensation alone is responsible for motor dysfunction of the hand/fingers in T2D, motor function in the anesthetized states of the control group should correspond to motor function of the baseline state in the T2D group. Alternatively, if another physiological mechanism is responsible for motor dysfunction, it can be expected that the T2D group will display greater reductions in manual function than the control group across all three testing conditions. Monofilament testing confirmed that tactile function in both WB and EB conditions in the control group were comparable to baseline tactile function in the T2D group. Analysis of timed clinical evaluations indicated that baseline function of the T2D group was similar to that of the control group under both anesthesia conditions. Linear and non-linear analysis of force production features was also performed. The data suggest that manual performance in patients with T2D may stem from a combination of tactile deficits as well as proprioceptive deficits.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

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Topic: D.17. Voluntary Movements

Support: Wellcome Trust 094874/Z/10/Z

James S. McDonnell Foundation, Scholar award

Title: Motor and sensory components of finger representations in the human brain

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Abstract: We used high-resolution functional MRI and multivariate analysis techniques to study the structure of cortical and cerebellar digit representations. To determine the influence of motor efference and sensory afference, we let subjects either produce isometric finger presses against a keyboard, or we moved their fingers passively using a pneumatic device. The force profiles experienced at the fingertips were approximately matched across active and passive conditions. Functional imaging data were acquired on a 7T scanner, using an isometric resolution of 1.4mm, 47 slices, TR=3s, and 8 runs with 107 images each. Each finger was moved 4 times during each trial, which lasted a total of 8.8 s. Each trial type was repeated 3 times during each run. The primary sensory cortex (Brodman area [BA] 3, 1, and 2), primary motor cortex (BA 4) and caudal premotor cortex (BA 6), the supplementary motor area, and lobules V / VI of the ipsilateral cerebellum revealed significantly digit-specific patterns. In cortical areas, the finger-specific patterns in the active and passive conditions correlated on average with r=0.79 across voxels with each other. This suggests that sensory information from each finger is transmitted to the cortical circuits that are involved in controlling movement of the digit. Both passive and active maps exhibited an invariant representational structure across regions and subject. While the underlying activity patterns themselves were variable, the relative overlap between the patterns was invariant. In contralateral sensory-motor areas, the passive condition elicited overall less activity than the active condition, but led to more distinguishable activity patterns. The ipsilateral sensory-motor regions were partly suppressed below baseline. However, for the active conditions a clear digit representation was found even in suppressed areas. This representation was less pronounced in BA 1 and 3, but stronger in BA 2, 4 and 6. Furthermore, the ipsilateral representation was much weaker during passive than during movement. Similar observations were made for the cerebellar digit representations. These results suggest that, while the contralateral digit representations observable in fMRI reflect mainly sensory re-afferent information, the ipsilateral and cerebellar digit representations are driven to a larger degree by an efference copy of the motor command.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

Location: Hall A

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Program#/Poster#: 709.17/U40

Topic: D.17. Voluntary Movements

Support: Doctoral Student Research Grant by Graduate center of City University of New York

Provost Research Scholarship by College of Staten Island / CUNY

Title: Effects of absent somatosensory feedback on digital isometric force control

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Abstract: Success skilled manipulation requires complex force coordination among the digits, which requires the CNS ability to integrate sensation feedback signals and descending motor commands (Gordon et al. 1993). Appropriate modulation of forces, either within- or across-digits, relies on responses triggered by tactile feedback (Macefield et al. 1996), as a result of perturbations (Hermsdorfer et al. 1999; Johansson et al. 1999) or when digit forces are erroneously planned (Edin et al. 1992; Flanagan and Wing 1997). Such short-latency force responses, however, are absent or delayed when the tactile sensation is blocked under digital anesthesia (Monzee et al. 2003). The present study was designed to examine how intact and absent somatosensory feedback modalities are integrated and contribute towards implementing digital force production, by selectively removing tactile feedback from the fingertips, and providing visual feedback of performance error during isometric finger force production tasks. Twelve (6 males, 6 female) healthy, right handed, young adults participated in this study. Four 6-dimentional force/torque sensors were installed horizontally on the table to measure individual finger force. All subjects were instructed to perform three isometric force production tasks: one maximal force task, and two template-tracing force production tasks (ramp-like and step-like). Force templates and individual's performance were displayed on a computer monitor during the experiment. All tasks were repeated before (Control group) and after a digital nerve block procedure (Anesthesia group) on subjects' index and middle fingers of right hand. We analyzed subjects' digital maximal force, force sharing patterns, enslaving effect, and task performance error in Anesthesia group vs. Control group. Our results showed that maximal force ability was reduced in anesthesia group not only at local digits (index, middle, and index-middle combination), but also at non-local digit (little finger). In addition, maximal force ability by all four fingers was maintained (significant interaction Group×Condition, $P < 0.05$) with individual force sharing pattern re-distributed, especially at non-local fingers (ring and little). However, no

group difference was found in either enslaving effect or root mean square error of force performance. These results suggest that sensory information from one digit is shared across other digits to attain and maintain task-specific performance stability, and thus absent tactile sensation feedback interrupts the maximal force ability and force sharing pattern not only at local but non-local fingers.

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Poster

709. Finger and Grasp: Age, Pathology, and Physiology

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Topic: D.17. Voluntary Movements

Support: The Royal Society

Wellcome Trust

Medical Research Council

Merton College, University of Oxford

Title: 7T fMRI reveals preserved SI topography of phantom fingers decades post amputation

Authors: *S. KIKKERT[1,3], J. KOLASINSKI[1], S. JBABDI[1], I. TRACEY[1,2], C. F. BECKMANN[3], H. JOHANSEN-BERG[1], T. R. MAKIN[1];
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Abstract: Sensory deprivation has been considered a major driving force for brain plasticity. However, both in human and animal studies this view is based on indirect evidence of emerging representations in the "deprived" cortex (e.g. studying representations of adjacent body parts in the cortical territory of the missing hand). This leaves a potentially latent topography of the deprived brain region unexplored. Recent research shows that lingering sensations of the missing hand ("phantom sensations") are associated with activity in the deprived hand region of amputees. It is unknown whether this reflects some preservation of functional representation, or rather reflects disorganized aberrant inputs. Here we studied organisation in the cortical territory

of the missing hand by taking advantage of the vivid phantom sensations individuals experience decades post arm amputation. We used ultra-high field 7T fMRI (1.2mm3 resolution) to investigate whether representation of the phantom hand is topographically organized. We asked two unilateral upper-limb (above and below elbow) amputees with exceptionally vivid kinaesthetic phantom sensations during phantom finger movements and twelve control participants to volitionally move individual phantom or intact hand fingers. Movements were executed in a phase-encoding paradigm and a block design. We identified a detailed topographic map of the phantom hand in both amputees, where neighbouring clusters showed selectivity for neighbouring phantom fingers in the primary somatosensory cortex (SI) contralateral to the amputation. This topographic representation was not significantly different from maps seen in matched control participants, as shown using a multivoxel pattern analysis, and was independent of the intact hand representation. No such representation emerged when the amputees imagined phantom finger movements (not resulting in vivid kinaesthetic sensations). To better investigate the relationship between the preserved finger maps and peripheral inputs, we also studied phantom finger topography in an amputee suffering from a brachial plexus avulsion (abolishing afferent inputs and efferent outputs to the residual limb) with good motor control over his phantom fingers. Using multiband 3T fMRI (2mm3 resolution), we identified preserved topography during phantom finger movements, suggesting that the observed SI topography does not reflect peripheral reorganisation. Our results reveal that organised use-related inputs are not necessary to preserve topographic mapping in the somatosensory cortex. Our results call for a reassessment of plasticity following cortical deprivation of input.

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Poster

710. Motor Learning: Mechanisms

Location: Hall A

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Program#/Poster#: 710.01/U42

Topic: D.17. Voluntary Movements

Support: 973 Program, 2011CBA00400

Strategic Priority Research Program, XDB02020001

Title: Changes in neuronal ensemble activities in the motor cortex during motor learning

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Abstract: *In vivo* two-photon imaging of a large ensemble of layer 2/3 pyramidal neurons of the mouse primary motor cortex, using viral transfection of GCaMP6, allowed us to monitor changes in neuronal ensemble activities during motor learning. Head-fixed mice were trained to respond to a sound cue by initiating a motor task of moving a bar laterally with one arm over a defined distance in order to receive a water reward, while imaging was made from the contralateral motor cortex. The same ensemble of neurons was monitored for their activities during the two-week period that the mouse learned to perform the task. For neurons that were active during the task (~100), we observed two types of changes for the learning period. First, firing pattern of each neuron became more temporally reproducible among trials. When aligned with the sound cue, these neurons were reliably firing at a defined time after learning (e.g., day 14). Furthermore, activities among most neurons were correlated with a temporal sequence and some neurons fired prior to the movement initiation. By contrast, this reproducible firing pattern was not observed in most neurons at the early period of learning (e.g., day 1-4). Second, in a minority of neurons, reproducible firing patterns were observed among trials at each day, but gradual forward shift of the pattern towards the sound cue was found with days of learning. These results indicate activity patterns of layer 2/3 motor neurons may reflect the process of learning motor sequence in the cortex and further studies of the mechanism underlying the temporal changes in firing patterns may help to dissect neural circuit basis of motor sequence learning.

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Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Title: Do consolidation of motor memory and interlimb transfer of adaptation rely on common processes?

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Abstract: When we perform a motor task, such as reaching for an object, under a novel condition, we first adapt slowly to perturbations. However, when exposed to the same perturbation after several hours or days, we adapt much faster. This “savings” phenomenon is thought to stem from the consolidation of motor memory. The amount of savings is predicted by a specific sub-component of adaptation known as the “slow process”. This process responds slowly to movement errors but retains information well from one trial to another. Its contribution also increases from the beginning to the end of the learning phase. Brain structures involved in the end of the learning phase were shown to characterize interlimb transfer of adaptation, i.e. when adaptation of one arm benefits to the opposite arm. We thus hypothesized that consolidation of motor memory and interlimb transfer of adaptation both rely on the slow process. To test this, we designed experiments in which subjects had to reach with their arm for visual targets in a rotating platform producing a velocity-dependent force field. Subjects were right-handed young adults. They had no visual, only proprioceptive feedback of hand movements. On the first 1, there were 3 experimental phases: pre-rotation (right/left hand), training (right hand) and post-rotation (left/right hand). We assessed interlimb transfer by comparing the baseline initial direction of the left hand with the first trial of the post-test. On the second day, we assessed consolidation of adaptation by testing again the right hand in the force-field. Preliminary results (N=5) indicate that there is a small but significant difference in initial movement direction (measured at peak velocity) between the pre- and post-rotation phase of the left hand, indicating interlimb transfer. Analyses of savings were not conclusive so far, but it is likely that testing more subjects will help determining whether there is a link between the amount of interlimb transfer and the amount of savings.

Disclosures: **H. Lefumat:** None. **P. Mutha:** None. **J. Vercher:** None. **C. Miall:** None. **F. Sarlegna:** None.

Poster

710. Motor Learning: Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.17. Voluntary Movements

Support: H2020-MSCA-ITN-2014

Title: Modulation of sensorimotor β-band activity by unilateral kinematic-errors in bimanual coordination tasks

Authors: J. ALAYRANGUES, F. TORRECILLOS, A. BROVELLI, *N. MALFAIT;
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Abstract: The functional significance of the EEG oscillations recorded over the sensorimotor cortex is still unclear. In a previous study, we examined the modulations in the β-band (15-30Hz) sensorimotor oscillatory activity in response to kinematic errors induced in unilateral reaching movements. We described the error-related attenuations of the post-movement β-ERS (β-rebound) at the end of perturbed reaches as well as the modulations in the β-activity during the preparation of the immediately following movements. The clearly distinct patterns of modulation that we observed for the pre- and the post-movement periods pointed to their different functional roles in behavioral monitoring and sensorimotor adaptation. While the error-related attenuation of the β-rebound may reflect salience-processing, independent of sensorimotor adaptation, the modulation of the pre-movement β-power might be related to motor-command adjustments activated after movement-execution errors are experienced. In the present experiment, we examine error-related modulations of β-activity in a bimanual reaching task. Our goal is to determine whether "high-level" information influences the patterns of interhemispheric asymmetry in pre- and post-movement β-activity. In this purpose, we use two tasks involving comparable bilateral reaching movements, but in which the mode of bimanual coordination differs critically: on the one side, a task of "parallel" coordination implicates the control of two independent cursors (each controlled by one hand) to reach two different targets, on the other side, a "cooperative" coordination task involves controlling a single cursor with both hands to hit a unique target. Unpredictable mechanical perturbations (force-field) are applied unilaterally, to one of the arm. As previously reported, in these two tasks different behavioral responses to unilateral perturbations are observed (Diedrichsen, 2007). In the task of parallel control, on-line motor correction and adaptive processes (visible in the movements executed directly after a perturbed trial) are manifest for the arm submitted to the perturbation solely. In contrast, in the cooperative condition in which a single cursor is controlled by both hands, correction during the perturbed movement and motor-command update in the subsequent trial are observed bilaterally. We assess how the error-related modulation of the sensorimotor pre- and post-movement β-activity (in particular the degree of interhemispheric asymmetry) is influenced by the nature of the bimanual coordination, parallel or cooperative.

Disclosures: J. Alayrangues: None. **F. Torrecillos:** None. **A. Brovelli:** None. **N. Malfait:** None.

Poster

710. Motor Learning: Mechanisms

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Program#/Poster#: 710.04/V3

Topic: D.17. Voluntary Movements

Support: KAKENHI #262174

KAKENHI #26242062

Title: Gain alteration of feedback control and adaptation induced by acquisition of a novel visuomotor map of reaching movements

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Abstract: Human motor system has two kinds of mechanisms for reaching movement corrections: (1) online correction within a trial (feedback control) and (2) offline correction across trials (adaptation). These two mechanisms contributed to perform accurate and consistent movements in the presence of noises and/or uncertainties. However, these corrections could be achieved involuntarily or implicitly, raising a fundamental question of why and how the motor system can correct movements in an appropriate direction to reduce the movement error. We hypothesize that this ability is associated with feedforward control based on visuomotor map that accurately transforms the visual target direction into the movement direction. Without such visuomotor map, the motor system would be confused about in which direction the movement is corrected. To test the causal link of the visuomotor map with online and offline corrections, we examined how these corrections were influenced when the visuomotor map was distorted. We tried to distort the map using the method in our previous study (Hirashima & Nozaki, Curr Biol 2012). When participants reached to one of two targets located clockwise (CW) or counter-clockwise (CCW) from a straight-ahead direction, a gradually increasing visual rotation was implicitly applied to the cursor representing the position of the participant's hand. CW and CCW visual rotations for the CW and CCW targets, respectively, implicitly made the movement directions more inward. Thus, after the adaptation to a certain amount of rotations, the executed movement directions became smaller (i.e., inward) than the target directions. Despite such distortion of visuomotor map, the movement direction to the central target (located straight ahead) remained unchanged, guaranteeing that this movement could be used as probe trials. We examined how a reaching movement to the central target was corrected online when the target suddenly jumped to other locations and offline in the next trial after experiencing a visual rotation. We found that the inward visuomotor map distortion decreased the gain of online and offline corrections. We also observed that when the visuomotor map was distorted outward, the gains of corrections increased. These changes were consistent with the changes of the movement corrections predicted by each visuomotor map distortion. These results support our hypothesis that both online and offline corrections reflect the properties of the visuomotor map, and also

illustrate possible close links between feedforward control, feedback control, and how they are modified by adaptation.

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Poster

710. Motor Learning: Mechanisms

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Program#/Poster#: 710.05/V4

Topic: D.17. Voluntary Movements

Support: Kakenhi A26242062

Title: Decomposing motor memory decay into trial- and time-dependent components

Authors: ***A. SASAKI**, D. NOZAKI;
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Abstract: Since a pioneering work of Ebbinghaus (1885), it has been repeatedly shown that declarative memory monotonically decays with passage of time. However, it has not been fully elucidated how the motor memory changes with the passage of time. Indeed, the current computational model for motor adaptation (state space model; e.g., Thoroughman & Shadmehr, Nature 2000) has succeeded in capturing the trial-dependent pattern of motor memory, but we have not yet known how the effect of passage of time should be included in this model. Here, we examined the time-dependent pattern of motor memory decay. Participants performed reaching movements in the presence of velocity-dependent rotational force field (FF) applied to the hand (Shadmehr & Mussa-Ivaldi, J. Neurosci. 1994). We used error-clamp (EC) method (Scheidt et al., J. Neurophysiol. 2000) in which the force exerted against a virtual channel was measured to quantify the level of motor adaptation (i.e., motor memory). After performing 100 FF training trials interleaved randomly with 25 EC trials, the participants repeated a test set 100 times. Each test set consisted of six FF trials followed by two consecutive EC trials. The inter-trial interval (ITI) between the two EC trials was changed variously (3, 4, 5, 6, 7, 8,1 9, 12, 18 or 24 sec) so that we could investigate how the motor memory decayed with the passage of time. It should be noted that the first EC trial could not be the FF trial: The FF trial would produce time-dependent learning effect (Huang & Shadmehr, J. Neurophysiol. 2007) and contaminate the motor memory decaying pattern. We observed a small amount of reduction of motor memory from 1st to 2nd EC trial when the ITI was 3 sec. This amount of motor memory was maintained until ITI became 8 sec before showing gradual reduction with longer ITI (> 8 sec). Thus, this decaying pattern was

different from a naive prediction that motor memory decayed monotonically with passage of time (e.g. exponential curve). These results suggest that motor memory decay can be separated into two different components: the component reducing the memory immediately after a performing a trial (trial-dependent component) and that decays with passage of time (time-dependent component).

Disclosures: A. Sasaki: None. **D. Nozaki:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: NSERC Grant

CIHR Grant

Title: Neuroplasticity in primary somatosensory cortex supports motor learning by observing

Authors: *H. R. MCGREGOR, P. L. GRIBBLE;
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Abstract: While many of our motor skills are acquired through physical practice, we can also learn how to make movements by observing others. A series of studies has demonstrated that individuals can learn how to reach in novel dynamical environments ('force fields', FF) by observing the movements of a tutor. Moreover, as has been shown in the literature on motor learning through physical practice, observational motor learning also brings about sensory-motor plasticity and performance changes in the somatosensory domain. Here we directly tested the involvement of primary somatosensory cortex (S1) in observational motor learning. Subjects grasped the handle of a robotic manipulandum and performed straight reaching movements to a visual target in a null field (no force applied). Subjects in a learning group (n=16) then watched a video of a tutor learning to perform straight reaches while the robot applied a leftward FF. Subjects in a control group (n=16) watched a video of a tutor reaching in an unlearnable randomly-varying FF. We examined S1 function immediately before and after observation through the measurement of somatosensory evoked potentials (SEPs). SEPs were elicited through electrical stimulation of the median nerve at the right wrist and were recorded over left S1. We quantified pre- to post-observation changes in the amplitude of the N20-P25 SEP

component, the first cortical component of the SEP which is generated by S1. Examination of the N20-P25 SEP component thus allowed us to assess changes in early, low-level S1 function associated with motor learning through observing. After acquiring SEPs, all subjects performed a behavioral motor learning test to assess the degree to which they had learned the observed FF. We show that subjects who observed motor learning exhibited increases in the N20-P25 SEP component measured over left S1 compared to the control group who observed a tutor performing curved reaching movements but not learning. Moreover, the increases in N20-P25 amplitude in the learning group were reliably correlated with the amount of motor learning subjects achieved through observation, as assessed behaviorally after SEP recordings. These results suggest that plasticity in primary somatosensory cortex supports motor learning by observing.

Disclosures: H.R. McGregor: None. **P.L. Gribble:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: Binational United States-Israel Science Foundation Grant 2011066

Title: Does representation of sensory delays in the motor system depend on the magnitude of the delay?

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[1]Ben-Gurion Univ. of the Negev, Beer Sheva, Israel; [2]Northwestern Univ., Chicago, IL; [3]Rehabil. Inst. of Chicago, Chicago, IL

Abstract: When we interact with the external world, our brain integrates various sensory inputs and maintains an internal representation of world dynamics. Due to the different physical properties and neuronal processing pathways of the inputs, sensory inputs are processed at variable delays with respect to their external source. Thus, our perception of multisensory events as simultaneous must be the outcome of active reconstruction processes that account for these delays. Here, we examine how the magnitude of the delay and the schedule of its presentation affect the representation of state and time in the motor system. To study the effects of delayed feedback on the performance of reaching movements, we used a virtual game of pong in which

the movement of the controlled paddle is delayed with respect to the movement of the participant's hand. Previous results indicated that for delays smaller than 150 ms, adaptation to the delayed environment also causes an overshoot in subsequent reaching to targets without vision of the hand (blind reaching). We suggested that these results can be explained by a state-based model in which a representation is based on a perceived change in the impedance parameters of the arm, rather than by a time-based representation model. In the current study, we explore whether a state-based representation of the delayed pong environment is bounded to small values of delay, and whether it is affected by the schedule of presentation of delay. We hypothesize that for large values of delay, the representation of the perturbation is more likely to be time-based. To test this, we exposed participants to delays of up to 300 ms, either by gradually increasing or decreasing the delay, or by presenting the participants abruptly with a constant perturbation of different delay magnitudes. During the experiments, participants also performed several blocks of blind reaches that were presented at constant intervals throughout the experiment, enabling us to examine the way they generalize their adaptation to the delay to a task that requires an accurate proprioceptive spatial representation. If large delays were explicitly compensated by a temporal representation, this representation would abolish the errors in blind reaching movements. Understanding the way delay is represented in the motor system is important for understanding how forward models are formed. It may also help in understanding pathological conditions that are characterized by delayed information transmission, such as Multiple Sclerosis.

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Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: Banting Post-doctoral Fellowship BPF-NSERC-01098

National Institute of Child Health and Human Development Grant R01 HD075740

Title: Learning novel sensory-motor maps: learning to move to auditory targets

Authors: *F. T. VAN VUGT[1], D. J. OSTRY[2];
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Abstract: Humans readily learn motor skills such as reaching to visual targets, learning to speak or learning to play a musical instrument. In such tasks, the brain faces a double challenge: it needs to learn which movement achieves a certain aim (e.g., which motor commands produce a given vowel; for feedforward control) and it needs to learn which motor corrections to generate for a given sensory error (for feedback control). We hypothesized that this knowledge is organised into two maps: a target and error map, respectively. Existing motor learning paradigms typically test participants who already have well learned, pre-existing maps that are perturbed experimentally, but it remains unclear how such maps are initially formed. We test human participants who hold a robot handle and make reaching movements to auditory targets. Movements elicit a combination of pure tones whose frequencies depend on the endpoint position of the handle in the horizontal plane. In experiment I, auditory targets are chosen quasi-randomly from a continuous distribution. In experiment II, targets are chosen from a set of five targets that are presented in random order. In experiment III, auditory targets are continuous but each target is presented 16 times in a row (batch). Presenting different targets on subsequent trials allowed us to probe the target map, whereas presenting the same target repeatedly tapped into the error map. that novel audiomotor maps can be readily acquired within a single session on a single day, as shown by decreased target-movement error. Performance on purely auditory and purely motor tests remained stable, showing that learning cannot be explained as a change in auditory or motor function but is attributable to the learning of the mapping between them. A very coarse target map of the sensorimotor space was acquired within a few trials and became progressively more fine-grained, allowing it to eventually encode at least five unique targets at the end of the session. When targets were presented in batches, participants improved their accuracy within a batch, and critically this within-batch convergence became faster in the course of the experiment, revealing that they learned an error map. These results show that novel sensorimotor maps can be acquired remarkably rapidly. Our paradigm allows monitoring the formation of two types of mapping: a mapping between motor commands and sensory outputs (target map), analogous to feed-forward control; and a mapping between sensory errors and motor corrections (error maps), analogous to feedback control. This experimental paradigm provides a unique window into the earliest stages of sensorimotor learning.

Disclosures: F.T. Van Vugt: None. **D.J. Ostry:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: NIH Grant GM103645

Title: Effective connectivity changes before and after sudden and gradual visual-motor adaptation

Authors: P. BÉDARD, *J. N. SANES;
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Abstract: Environmental changes occur constantly to form and then retain new sensory-motor relationships. We previously examined differences in brain activation and effective connectivity (EC) during adaptation to and retention of sudden and gradual perturbations (Bédard and Sanes 2014). Here, we assessed whether adapting to varying visual-motor regimens differentially changed spontaneous brain states (Albert et al. 2009). We used functional MRI to measure brain activation from young adults who used a joystick to acquire visual targets. The visual cursor followed the joystick normally (null), was rotated 30° on all trials (Sudden), or rotated incrementally 0.25°/trial, starting at 0° (Gradual). No one in the Gradual group became aware of the perturbation. All participants performed six phases: Null (80 trials), Rest (8 min), Learning (160 rotation trials), Washout (80 null trials), Rest (8 min), Recall (80 rotation trials at 30°). We analyzed EC during the two Rest periods using Granger causality methods between 10 regions that exhibited common activation across the two groups late in Learning. Reaching error of the Sudden group steadily decreased during Learning, while error for the Gradual group steadily increased to approximate that of the Sudden group late in Learning. Both groups showed similar performance during Washout and Recall. For the EC analyses, we found more directed influence in the Gradual group by the left superior parietal lobule (SPL) on the right cerebellum (CB) and by left premotor cortex (PM) on the left occipital gyrus after compared to before Learning. By contrast, the Gradual group exhibited less influence by the right SPL on medial CB and also on the left middle occipital gyrus and also less influence by the left PM on the right CB after compared to before Learning. For the Sudden group, we found more influence by the left somatic sensory cortex (S1) on medial CB and also by the right SPL on the left S1 after compared to before Learning. When directly comparing the Sudden to the Gradual group before Learning, we found more influence from left SPL to the middle CB for the Sudden group. After Learning, we found more influence from right SPL to left S1 and from left S1 to the right CB for the Sudden group and finally, more directed influence from the left SPL to the right CB for the Gradual group. These results demonstrated short-term changes in network connectivity, independent of task performance and also independent of the apparent similarity in learning two different types of adaptation, as measured by performance during Washout and Recall for the two groups. Thus, it appears that motor memory consolidation uses different brain mechanisms for different tasks.

Disclosures: P. Bédard: None. **J.N. Sanes:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: National Institute of Child Health and Human Development R01 HD075740

Title: Neural substrates of the initial stages of human motor learning

Authors: *A. SIDARTA, N. BERNARDI, S. VAHDAT, D. J. OSTRY;
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Abstract: The initial stages of motor learning are often characterized by uncertain sensory goals. In learning how to play tennis, for example, one has a distal visual target that helps constrain the movement. However, it is necessary to discover, in somatosensory terms, what makes a good serve in the absence of direct visual guidance. During this period, motor commands and associated sensory targets are acquired and refined through trial, error and exploration. Reinforcement-based feedback, both implicit and explicit, can serve as a means to shape motor behavior. In this study, we focus on the functional brain networks that are involved in the initial period of motor learning. We recruited 21 healthy right-handed subjects who performed active reaching movements to a horizontal bar that contained a unseen target zone. During training trials, subjects were given feedback if their movement was accurate, that is, if it landed inside the reward zone. The size of the reward zone was progressively reduced across the four training blocks (50 trials each). Multiband-accelerated fMRI recording (TR=1.69sec, voxel size: 2mm isomorphic) was used under resting-state conditions, immediately before and after movement training. Seed-based analyses were conducted using a set of region of interests (ROIs) from the task-based localizer. We included as regressors in a group-level GLM, the reduction in movement error and the number of reinforced movements, to test whether changes in functional connectivity were associated with behavioural measures of learning. We found a reliable improvement in movement accuracy after the reinforcement-based training. This improvement was reliably correlated with the amount of reinforcement received. We also found that there was an increase in functional connectivity strength within the sensorimotor networks. In particular, networks involving supplementary motor area (SMA), primary and second somatosensory cortices, and the ventromedial prefrontal cortex show reliable increases associated with accuracy improvement following learning. This suggests that early motor learning involves changes to somatosensory as well as motor networks which occur in conjunction with reinforcement.

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Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: FIS/IMSS/PROT/G11-2/1028

Title: Plastic changes in dendritic spines of pyramidal neurons from prefrontal cortex underlie motor learning in rats

Authors: ***M. N. VÁZQUEZ HERNÁNDEZ**[1], D. GONZÁLEZ-TAPIA[2], N. I. MARTÍNEZ-TORRES[1], M. HERNÁNDEZ-GONZÁLEZ[3], I. GONZÁLEZ-BURGOS[1];
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Abstract: The ability to develop new skills through practice is defined as motor learning. The prefrontal cortex is involved in the rectification of information related to the achievement of motor abilities to be learned. Particularly, pyramidal neurons of the layer V of prefrontal cortex receive excitatory information previously integrated in the cortical-striatum-thalamic pathway, for its rectification. Excitatory synaptic inputs of these neurons are mainly mediated by dendritic spines, which regulate the income of synaptic stimulation that arrives to these neurons. In the present study, numerical density of dendritic spines and spine type proportions of pyramidal neurons of prefrontal cortex were investigated to help to elucidate the plastic changes underlying synaptic information processing during the period in which motor skills are acquired. Six Spregue-Dawley adult male rats where assigned to one of six sub-groups of study, according to a six-day paradigm of motor training. 36 non-trained rats, and 6 intact rats where used as controls. The time elapsed to traverse the trail, and the number of errors committed per session (four trials per day), were used as indicators of motor learning. Then, the prefrontal cortex of experimental animals where dissected out for quantification of numerical density and spine type proportions by direct observation under a light microscope. Pyramidal neurons showed more dendritic spines on days 1 and 3, as well as more thin spines on day 1 and less mushroom spines on days 2, 5 and 6. The findings allow to suggest that the rectifying participation of prefrontal cortex during motor learning is predominant on the first days of training based on the elevated number of thin spines on these days. According with this, since thin spines have been associated whit the

acquisition of novel information whilst mushroom spines are rather related with its long-term storage, the greater number of thin spines observed could indicate that the rectifying processes leading to motor learning may imply a rapid processing of information in the prefrontal cortex.

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Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: CHDI Foundation

Title: Representation of reaching movements in motor cortex: a modeling perspective

Authors: ***W. W. TEKA**[1], K. HAMADE[2], S. N. MARKIN[2], R. F. ROGERS[3], I. A. RYBAK[2], Y. I. MOLKOV[1];
[1]Indiana Univ. – Purdue Univ. Indianapolis, Indianapolis, IN; [2]Drexel Univ. Col. of Med., Philadelphia, PA; [3]CHDI Mgmt., Princeton, NJ

Abstract: The motor cortex plays a major role in controlling voluntary movements by sending signals to the muscles through the spinal cord. Because of mapping complexity, the cortical representation of movement, and the relationship between the cortical neuronal activity and the dynamics of movement are not fully understood. How different movements are coded and represented in the cortical activity is a central motor control problem. Previous studies were based on two competing hypotheses. One approach attempts to correlate activity of primary motor cortex neurons during reaching movements with movement parameters (e.g., direction). However, these correlations cannot prove causation, and the directional tuning of given neuron may depend on other factors. A different approach suggests a dynamical paradigm of motor control based on the suggestion that neural activity in the motor cortex represents motor programs corresponding to a particular motor task (e.g., moving an arm from one position to another to reach an object) rather than the parameters of the movement per se. We have developed a model of a neuronal controller which governs a two-joint arm actuated by six muscles. This model describes the dynamics of voluntary arm movement controlled by a spinal cord circuit with afferent feedbacks. A thalamo-cerebellar-cortical controller in the model provides the neuronal motor program necessary to perform reaching tasks based on a bell-shaped

velocity profile and a straight line trajectory. To do so the controller calculates the joint torques which are necessary to move the arm along the specified trajectory and then generates these torques with agonist and antagonist muscles via specific activation of the low-level spinal circuit. The model was used to evaluate the relationships between neuronal activity in primary motor cortex, muscle activity and arm dynamics and to compare them with those reported in the previous experimental studies. The model demonstrates that patterns of activity in the motor cortex may indeed strongly correlate with both dynamical and geometric parameters of the movement. However, the apparent moto-cortical "coding" of a particular parameter depends on the experimental context, which might not be taken into account or reported. On the other hand, afferent feedback signals from muscles during the movement include information about movement geometry, and these signals are factored into the calculation of the motor program. We conclude that the dynamical paradigm of cortical representation of movement implies coding of both dynamical movement parameters and its geometrical characteristics.

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Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01HD073147

Title: Difficulty of visual transformation modulates the contributions of explicit and implicit learning with and without tDCS

Authors: *S.-L. LIEW[1], T. THOMPSON[2], J. RAMIREZ[2], P. BUTCHER[3], J. TAYLOR[3], P. CELNIK[2];
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Abstract: Recent research has shown that both error-based and strategy-based learning play a role in visuomotor adaptation (Taylor et al., 2014). Error-based learning involves the implicit generation of an internal model to predict movement errors, while strategy-based learning engages explicit instructions or cognitive strategies to improve motor learning. In the present study, we examined the effects of task difficulty on the contributions of error-based and strategy-

based learning. To do so, we increased the amount of visual transformation by changing the feedback screen orientation from horizontal to vertical. We predicted that this would increase the task demand on participants by adding a new level of visuomotor transformation. We collected data in two groups of participants (n=10 per group) who performed the exact same task with either a horizontal or vertical screen. Using the same visuomotor adaptation paradigm as Taylor and colleagues (2014), participants directed a stylus towards a target on a circle, flanked by numbers, presented on a computer screen. Participants were asked to try to get their cursor on the target, and also to verbally report the number they were aiming at in order to get their cursor on the target. In this way, both explicit (aiming report) and implicit (movement trajectory) components of each movement were measured. Participants were unable to see their hand during the task. After 120 baseline trials, a 45-degree clockwise rotation was introduced for 160 trials, followed by a block of 40 trials without feedback and 40 trials with feedback. We found that the vertical group performed worse than those in the horizontal group, showing a slower rate of learning. While both groups used the same amount of error-based learning, the vertical group used less of an aiming strategy compared to horizontal group. That is, increasing the task difficulty through the screen orientation modulated the relative contribution of the explicit aiming component during the visuomotor adaptation task. With this version of the task, we also replicated findings from a previous study using transcranial direct current stimulation. We found that facilitation of the cerebellum by anodal tDCS greatly increased the error-based learning component, while facilitation of the dorsolateral prefrontal cortex greatly increased the strategy-based learning component, relative to sham. The effects of tDCS on these explicit and implicit components were increased when the task was more difficult (vertical screen). These findings suggest that task difficulty via visual transformations can affect the contributions of implicit and explicit components during visuomotor adaptation.

Disclosures: **S. Liew:** None. **T. Thompson:** None. **J. Ramirez:** None. **P. Butcher:** None. **J. Taylor:** None. **P. Celnik:** None.

Poster

710. Motor Learning: Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 710.14/V13

Topic: D.17. Voluntary Movements

Support: NSERC

Title: Concurrent reach and tracking adaptations of moving and static targets

Authors: *M. N. AYALA, P. SHARMA, D. Y. P. HENRIQUES;
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Abstract: Given that the neural networks and behavioural parameters subserving saccadic and smooth pursuit eye movements are independent of one another, we wanted to explore whether a similar analogy exists for ballistic reaching and tracking arm movements. Does adaptation to perturbed tracking movements generalize to that of ballistic reaching movements? In the following experiments, we explored whether training by tracking a moving target with a perturbed hand-cursor produces motor aftereffects and if these aftereffects differ from those produced in a typical perturbed ballistic reaching task with a static target. We found that adaptation to perturbed tracking movements produce significant reach aftereffects although to a smaller extent; tracking aftereffects were about half the size (on average 9°) of those produced after ballistic reach training (on average 19°). Additionally, we looked at whether neural processing of adaptation to tracking and reaching paradigms are independent of one another and would thus allow for concurrent adaptation to opposing perturbations (i.e. dual adaptation). Tracking trials were associated with a 30° CCW rotation while reach trials were associated with a 30° CW rotation. The 'single' perturbation groups adapted to either a CW or CCW perturbation while the 'dual' group experienced both perturbations concurrently. We found significant reach aftereffects following dual training of about 7°, which was substantially smaller than that produced when reach training was not concurrent with tracking training. The size of reduction of aftereffects is consistent with the extent of the interference from tracking training as measured by the reach aftereffects produced when only that condition was performed. Additionally, tracking performance in response to a visuomotor rotation significantly improved across training for both single and dual tracking groups, both saturating at the same level but with different learning curves, with only the single group fully returning to baseline levels. Finally, reach errors for static targets in the dual group significantly decrease across training although to a lesser extent when compared to that of the single group. These findings suggest that adaptation of tracking movements which tend to produce small errors that can be adjusted on-line, are processed somewhat, although not completely independent of reaching movements which tend to produce larger errors that are adjusted on a trial-by-trial basis.

Disclosures: **M.N. Ayala:** None. **P. Sharma:** None. **D.Y.P. Henriques:** None.

Poster

710. Motor Learning: Mechanisms

Location: Hall A

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Program#/Poster#: 710.15/V14

Topic: D.17. Voluntary Movements

Title: Individual differences in TMS sensitivity influence the effect of tDCS in a motor adaptation task

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Abstract: Transcranial direct current stimulation (tDCS) provides a non-invasive brain stimulation tool to induce change in cortical excitability and perturb or enhance motor and cognitive function. To date, tDCS researchers have employed a fixed stimulation level, ignoring the potential impact of individual anatomy and physiology on the efficacy of the stimulation. This fixed approach contrasts with the standard procedure for transcranial magnetic stimulation (TMS) where the stimulation level is usually tailored on an individual basis. Labruna et al. (submitted) reported a modest relationship between individual differences in sensitivity to TMS and the efficacy of anodal tDCS: Individuals with a lower threshold for TMS showed a larger change in corticospinal excitability following anodal tDCS, compared to individuals with a higher threshold. In the present study we examined the impact of this relationship on tDCS-induced effects on sensorimotor learning. As an operational measure of TMS sensitivity, we identified each participant's resting motor threshold, defined as the minimal TMS intensity required to evoke motor evoked potentials on 5 of 10 consecutive trials in the right index finger. Participants were then tested on a visuomotor adaptation task in which they had to reach to visual targets with the right hand. Following a baseline phase with veridical feedback, a 30-degree clockwise rotation was imposed for 200 trials. Retention and forgetting was tested in a final phase of 288 trials in which feedback was removed. Anodal or sham tDCS (1 mV) was applied over left M1 during the second half of the baseline phase and throughout the adaptation phase. Contrary to the results of Galea et al. (2011), acquisition was faster for participants receiving anodal tDCS compared to sham stimulation, whereas performance was similar for the two groups in the forgetting phase. Of greatest interest in the present study, the enhanced acquisition effect was larger in participants with lower TMS thresholds (higher sensitivity to TMS). These results suggest that TMS sensitivity can be used as predictive measure of tDCS efficacy. Moreover, customizing tDCS stimulation levels to account for individual differences in brain stimulation sensitivity may increase the reliability of this method.

Disclosures: L. Labruna: None. **M.J. Dabit:** None. **B. Vanderschelden:** None. **A. Stark-Inbar:** None. **M. Nitsche:** None. **R.B. Ivry:** None.

Poster

710. Motor Learning: Mechanisms

Location: Hall A

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Program#/Poster#: 710.16/V15

Topic: D.17. Voluntary Movements

Title: Distinct modulations in sensorimotor post- and pre-movement β-synchronization related to error salience processing and sensorimotor adaptation

Authors: *F. TORRECILLOS, J. ALAYRANGUES, B. E. KILAVIK, N. MALFAIT;
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Abstract: It is well known that β-band (15-30Hz) oscillations often dominate in sensorimotor cortex, however their functional roles remain uncertain. Prominent at rest, they were originally attributed to an "idling" state of the motor system. Along these lines, β oscillations within and beyond sensorimotor cortex were recently proposed as an active process that promotes the existing motor state and compromises neuronal processing of new movements. Recently, Tan et al. (J Neurosci 34:5678, 2014) examined sensorimotor β-band activity during a joystick task in which visual perturbations were introduced. They found that the β-rebound, an increase in β-power typically observed at the end of movement, was attenuated in trials with movement-execution errors. Furthermore, the effect was stronger when contextual information enhanced the behavioral salience of the error. Here, we investigated further the modulations in β-activity by movement errors, contrasting error salience and sensorimotor adaptation. In two complementary experiments, along with the post-movement β-rebound we examined the pre-movement β-band activity during preparation of reaches immediately following perturbed movements. In the first experiment, participants performed reaching movements in a force-field created by a robotic device, and kinematic errors of different sizes were produced by unpredictable changes in the strength of the applied force. In the second experiment, we contrasted two types of reach errors: movement-execution errors induced by mechanical or visual perturbations that trigger trial-to-trial adaptive mechanisms, and goal errors caused by unexpected displacements of the target that do not elicit sensorimotor adaptation. Consistent with Tan et al, we found that the post-movement β-rebound was parametrically attenuated by the sizes of movement-execution errors produced by mechanical perturbations independently of their corrections on-line (Exp1). Importantly, this error-related attenuation of the post-movement β-rebound appears insensitive to the nature of the reach errors, as it was present for both movement-execution and goal errors (Exp2). In comparison, the pre-movement β-activity presented a clearly contrasting pattern, only being modulated by kinematic errors that triggered trial-to-trial motor-command updates. Our findings suggest that the error-related modulation of the β-rebound may reflect salience-processing, independent of sensorimotor adaptation, whereas the attenuation of pre-movement β-

power might be related to motor-command adjustments activated after movement-execution errors are experienced.

Disclosures: F. Torrecillos: None. **J. Alayrangues:** None. **B.E. Kilavik:** None. **N. Malfait:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

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NINDS P01 NS044393 (PLS)

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VA (PLS)

Title: Effect of protein synthesis inhibition in monkey primary motor cortex on performance of sequential movements

Authors: *M. OHBAYASHI[1,2,3], P. L. STRICK[1,2,3,4];
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Abstract: Regular practice enables motor skills to be retained and improved. Recent studies in monkeys indicate that extended practice on a motor skill alters patterns of neural and functional activation in M1 (Matsuzaka et al., ; Picard et al.). These and other results have led to the notion that M1 is a site of storage for highly practiced skilled movements. Experiments in rodents support this conclusion and indicate that constitutive protein synthesis in M1 is required to maintain a learned motor skill (Kleim et al., 2003). Here we tested whether protein synthesis in M1 of monkeys is required for the maintenance of learned sequential movements. We trained 2 monkeys (Cebus Apella) to make sequential reaching movements in two task modes (Matsuzaka et al., 2007). In the Random mode movements were guided by vision and new targets were filled using a pseudo-random sequence. The new target in the sequence was filled 100 ms after contact of the prior target. In the Repeating mode movements were internally guided and new targets were filled according to a repeating 3 element sequence (e.g., 5-3-1-5-3-1 ...). A 400 ms delay

was inserted between contact of one target and the fill of the next target in the sequence. The longer delay promoted the occurrence of predictive responses and we allowed the animal to contact the next target in the sequence before the target was filled. With practice, monkeys could perform the Repeating mode of the task without any visual cues. After the monkeys were trained for more than 200 days on the Random and Repeating modes of the task, we injected the protein synthesis inhibitor anisomycin (ANI, 5 µl of 100 mg/ml) into 2 sites of the arm area of M1 (monkey 1, n = 5; Monkey 2, n = 1). One day later we tested the animals' performance on the two modes of the task. After the ANI injections, the number of errors increased significantly only during performance of the Repeating mode of the task. The error rate for the most affected movements increased by an average of 49.7% (range of 77-29%) after the ANI injections. Similar increases in errors were not observed during the Random mode of the task or after control injections of saline into M1 (n = 2). The errors during the Repeating mode can be categorized as two types: errors of accuracy and errors in direction. An accuracy error was considered to be a reach performed in the correct direction, but to an endpoint outside of the correct target. A direction error was considered to be a reach performed in the direction opposite to the correct target. Substantial number of the errors (an average of 57.3%, range of 97.6-8.3%) during the Repeating mode were direction errors. These results support the concept that M1 is a site of storage for highly practiced motor skills.

Disclosures: M. Ohbayashi: None. **P.L. Strick:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: NINDS Grant NS052236

Title: Task intuitiveness and non-linear filtering of surface electromyography facilitate learning of proportional 3D position control of a myoelectrically-controlled robot

Authors: *A. FEINMAN, T. D. SANGER;
USC, Los Angeles, CA

Abstract: In previous work, our group has shown that subjects can more easily control stable surface electromyography (sEMG) signal amplitudes when using Bayesian-filtered sEMG. In this study, we have begun to examine the factors that affect Fitts'-Law movement times and error

rates when subjects are required to control trajectories to acquire targets. Subjects were given control of the endpoint of a robotic arm. Endpoint position in Cartesian space was proportional to sEMG amplitudes of the flexor carpi radialis (FCR) for lateral motion, brachioradialis (BR) for upward motion, and anterior deltoid (AD) for forward motion. In each trial, a pair of bars was presented on the surface of a table in front of the subject, and they were asked to repeatedly tap the robot endpoint back and forth between the two bars as quickly as possible without missing. The two bars were identical in shape in each trial, but the width of the bars and/or the distance between the bars varied across trials to generate six indices of difficulty in the range of 1-4 bits. Five blocks of trials were conducted: 1 = tapping while grasping the robot, 2 = controlled robot using Bayesian-filtered sEMG, 3 = linear filtering, 4 = Bayesian filtering with FCR and BR Electrodes swapped, 5 = identical to block 2. Differences in movement times (MTs) and error rates (ERs) were observed. In blocks 2 & 3, where the action directions of the muscles matched the movements of the robot, subjects were able to achieve the correct motions in a matter of seconds. MTs did not improve between blocks 2 and 3, but ERs were higher in block 3. Subjects were mostly able to achieve a cyclic tapping in a short period of time. In block 4, most subjects could not learn the task quickly. Subjects that could learn the flipped task required more time to minimize ER and still had high MTs than in the intuitive task of the other blocks. Comparison between blocks 2 and 5 showed reduced MT and ER in block 5, despite subjects' having been exposed to different filtering and control paradigms in blocks 3 and 4, This indicates that subjects may be learning something that applies to that paradigm despite the interference. Our results show that both task intuitiveness and the type of EMG filtering affect the ability to learn the task quickly (i.e., minimize ER) and to master it (i.e., minimize MT). Future work in this project will extend to examining which kinematic states (or combinations) are the ideal control paramters, as well as studying the effect of visual aids on facilitating learning.

Disclosures: **A. Feinman:** None. **T.D. Sanger:** None.

Poster

710. Motor Learning: Mechanisms

Location: Hall A

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Program#/Poster#: 710.19/V18

Topic: D.17. Voluntary Movements

Support: CIHR

NSERC

FQRNT

Title: The role of dorsolateral prefrontal cortex in motor learning during force-field adaptation: A continuous theta-burst stimulation study

Authors: *K. TREWARTHA, J. P. GALLIVAN, J. FLANAGAN;
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Abstract: Many studies have investigated motor learning by requiring participants to learn to adapt to either visual (visuomotor rotation) or mechanical (force-field adaptation) perturbations. Two learning processes have been identified that support such learning: a fast process that adapts quickly, but also decays quickly; and a slow process that adapts and decays more gradually. The neural mechanisms underlying the fast and slow processes remain unclear. However, recent evidence has linked the fast process for motor learning to working memory resources that may be supported by dorsolateral prefrontal cortex (dlPFC). The current study used transcranial magnetic stimulation (TMS) in a continuous theta burst stimulation (cTBS) protocol to test the prediction that dlPFC plays an important role in the fast process for motor learning during force-field adaptation. Forty-eight healthy participants (18-34 years old) were recruited and assigned to one of three groups: a group receiving cTBS over right dlPFC, a group receiving cTBS over the arm area of left motor cortex (M1), and a control group who received no TMS. For the two cTBS groups, a 40 second train of pulses at an intensity of 80% of each participant's own active motor threshold was administered 5 minutes before participants completed a force-field adaptation experiment. cTBS is known to temporarily disrupt the function of the targeted brain area. On the group level our results were consistent with the hypothesis that temporary disruption of dlPFC selectively impairs the fast process for motor learning, leaving the slow process relatively intact. Behaviorally, participants in the dlPFC group exhibited slower adaptation during early stages of adaptation compared to both the M1 and no-TMS control groups. No differences were observed during later stages of adaptation, during a subsequent counter-adaptation phase, or during an error-clamp phase that immediately followed the counter-adaptation phase. No differences were observed between M1 and no-TMS control participants. These results are consistent with previous functional MRI data showing increased right dlPFC activity during early, but not late stages of visuomotor adaptation. The current data add to this literature showing that similar cognitive mechanisms are involved during early stages of learning during force-field adaptation. These findings suggest that the fast process for motor learning is highly cognitive, involving working memory resources that are supported by right dlPFC.

Disclosures: K. Trewartha: None. **J.P. Gallivan:** None. **J. Flanagan:** None.

Poster

710. Motor Learning: Mechanisms

Location: Hall A

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Topic: D.17. Voluntary Movements

Support: NIH Grant NS078311

Title: The feedback response to error is a teaching signal during motor adaptation

Authors: *S. T. ALBERT[1], R. SHADMEHR[2];
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Abstract: When performing a motor task, we update our feedforward (FF) motor commands on a trial-to-trial basis in order to correct for errors we experienced on the previous trial. Feedback (FB) motor commands generated on-line to correct for error may serve as a teaching signal which instructs this motor learning process. If error-based learning is truly instructed by FB motor commands, these commands should be correlated. We tested this hypothesis by measuring FB motor commands and isolating the learning that was induced by them in FF motor commands. To assay FF and FB motor commands, we used electromyography (EMG) to measure the motor output of various arm muscles during a center-out reaching task (n=57 subjects). Before and after each perturbation, the subject experienced an error-clamp trial. In error-clamp, the robot generates a stiff channel which compensates for forces produced perpendicular to the straight line path connecting the starting position and target. During error-clamp, we assume that the desired state of the limb exactly matches its actual state, thus eliminating FB updates. Under this assumption, the error-clamp EMG signal is a direct measure of the FF motor command. Therefore, the difference between the pre- and post-perturbation error-clamp EMG signals is a timecourse that represents learning. Our proxy for error-dependent feedback commands was the difference between the EMG signals measured during the perturbation and the preceding error-clamp trial. Under this framework, we computed mean learning and FB timecourses across subjects and noted that they were related by a temporal shift. Cross-correlation indicated that learning and FB commands were maximally correlated if the FB traces were shifted earlier in time by 140 ms, on average. To further investigate the correlation between learning and FB we artificially shifted the FB responses within-subject to align them with the learning traces, according to the cross-correlation. We determined temporal segments where shifted FB and learning were positively correlated by linearly regressing, across subjects, learning at a particular time point onto the shifted FB response. Using this analysis, we determined that learning and shifted FB were significantly correlated during at least 80% of movement duration. These data suggest that feedback motor commands serve as a template or teacher for the motor learning system. Trial-to-trial motor learning may be partly driven by the addition of time-shifted, reduced-amplitude FB responses to the previous FF motor command.

Disclosures: S.T. Albert: None. **R. Shadmehr:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: RO1 HD053793

JSPS KAKENHI (Grant-in-Aid for JSPS fellows, 25-4917)

Title: Occlusion of LTP-like plasticity in the primary motor cortex following adaptive motor learning

Authors: *S. UEHARA[1,4,5], F. MAWASE[1], P. CELNIK[1,2,3];
[1]Dept. of Physical Med. and Rehabil., [2]Dept. of Neurol., [3]Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD; [4]Ctr. for Information and Neural Network,, Natl. Inst. of Information and Communications and Technol., Osaka, Japan; [5]Japan Society for the Promotion of Sci., Tokyo, Japan

Abstract: When we learn a new motor behavior, different mechanisms are engaged including error-based (i.e. due to prediction errors), use-dependent (i.e. bias from prior movements), reinforcement (i.e. bias toward movements resulting in rewards) and strategic learning (i.e. due to cognitive strategies). These mechanisms seem to depend on different neural substrates, and importantly all of them are likely to contribute when learning new motor behaviors, even though some might be more weighted than others. An approach to test this framework is to determine whether physiological markers of one brain region are present during learning of behaviors. For instance, occlusion of LTP-like plasticity in M1 can be used as a physiological signature of motor memory retention (Cantarero 2013). Here, we tested whether learning a visuomotor adaptation task, known to heavily but not exclusively rely on error-based learning (presumably dependent on the cerebellum), also engages occlusion of LTP-like mechanisms in M1. Twenty subjects performed a visuomotor adaptation task involving reaching with their right index finger. In two counterbalanced sessions, all subjects were exposed to a 30-deg constant visuomotor perturbation and in the other session they were exposed to random perturbations. Half of the subjects performed 56 perturbed trials (short training group), while the other half performed 208 perturbed trials (long training group). Excitability of M1 was evaluated using single pulses of TMS before and after applying anodal transcranial direct current stimulation (AtDCS) to the left M1 for 7 min. To explore the effect of training on occlusion of LTP-like plasticity, we assessed the tDCS effects in a baseline session (no training session) and immediately after training. We

found that the short training group experienced an increase in M1 excitability by AtDCS at baseline and after training of both constant and random perturbations. However, the long training group had no increase in M1 excitability by AtDCS only after exposure to the constant perturbation, a signature of occlusion of LTP-like plasticity, whereas this effect was not present at baseline and after the random perturbation. Additionally, the subjects who leaned more, as depicted by more errors during catch trials, were those who showed larger occlusion effects. These findings indicate that M1 is engaged during visumotor adaptation learning paradigms if enough exposure is allowed, suggesting the potential presence of reinforcement or use dependent learning mechanisms underlying this behavior.

Disclosures: **S. Uehara:** None. **F. Mawase:** None. **P. Celnik:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.17. Voluntary Movements

Support: 9R01NS092079-20

Title: Reaching for a good aiming strategy in people with cerebellar degeneration

Authors: *R. MOREHEAD[1], J. A. TAYLOR[2], R. B. IVRY[1];
[1]Psychology, UC Berkeley, Berkeley, CA; [2]Psychology, Princeton Univ., Princeton, NJ

Abstract: In visuomotor adaptation tasks, a systematic discrepancy is introduced between visual feedback of the hand position and the actual position of the hand. Patients with ataxia from cerebellar degeneration show a marked deficit in their ability to compensate for these perturbations, an impairment attributed to an inability to utilize sensory prediction errors. However, it is increasingly recognized that behavior in visuomotor adaptation tasks reflects the contribution of other learning processes in addition to adaptation. We performed a several experiments to assess the impact of cerebellar ataxia on these different learning processes. Experiment 1 assessed aiming strategy use by associating an arbitrary color cue with a visuomotor rotation. Prior work has shown that internal models cannot be selectively retrieved by color cues, while different actions (where to point) are easily associated with such cues. For the latter, healthy controls can use the cue to retrieve and implement an appropriate aiming plan, flexibly changing this plan from trial to trial. We manipulated the color cue to assess how much of the performance change observed in an adaptation task is attributable to aiming. Patients with

cerebellar ataxia (n=25) performed this task with either one or four targets arrayed in 90° increments around the workspace. In the single target condition the patients performed the task remarkably well, showing a clear ability to develop and implement an aiming strategy to compensate for the perturbation. In contrast, in the four target condition, the patients were unable to develop and implement an aiming strategy. Experiment 2 employed a different manipulation thought to isolate error based adaptation. Here we used a visual error clamp in which the feedback was always offset from the target by 45°, independent of the direction of the hand movement. Participants (n=10) were tested with either one straight ahead target or four targets located around the workspace. Participants were told to move the unseen hand directly to the target location, ignoring the cursor. Despite these instructions, control participants deviate their hand path, suggestive of adaptation. Surprisingly, the patients showed similar changes in performance in the one target condition and, based on preliminary results, a modest attenuation in the four target condition. This discrepancy between the 1- and 4-target conditions in both experiments suggests that part of the performance deficit observed in patients with ataxia may extend beyond an impairment in adaptation.

Disclosures: R. Morehead: None. **J.A. Taylor:** None. **R.B. Ivry:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.05. Visual Sensory-motor Processing

Support: CNRS - PEPS

ANR - GRASP

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Title: Contrasting roles of distinct LFP beta bands in motor cortex and their relation to spiking activity

Authors: *B. E. KILAVIK, A. RIEHLE;
INT, CNRS - Aix-Marseille Univ., Marseille, France

Abstract: Sensorimotor beta oscillations (~ 13-30 Hz) modulate rather heterogeneously across different sensorimotor behaviors. Many roles in cognition and motor control were therefore proposed, including postural maintenance, signal expectancy and sensorimotor integration (Kilavik et al. 2013, Exp Neurol 245:15). In the motor cortex, local field potential (LFP) beta oscillation amplitudes and neuronal firing rates change systematically in relation to behavior, but their direct amplitude inter-dependence was barely studied. Furthermore, spikes lock to beta oscillation phase, but it is unclear whether spikes of single neurons lock to multiple rhythms co-occurring in the LFP, and if the locking is dependent on the neuron's task selectivity. We studied motor cortical LFP beta oscillations and neuronal spiking activity in macaque monkeys performing delayed visuomotor reaching tasks. Beta oscillations modulated systematically in relation to the behavioral context, in particular when comparing visual cue anticipation, cue processing and movement preparation. Importantly, not only beta power, but also beta frequency changed systematically with behavioral context, and sometimes two bands were observed simultaneously (below and above 20 Hz). Along the behavioral trial LFP beta amplitudes and multi-unit activity levels were negatively correlated, but within specific task epochs, significant across-trial correlations were rare. Furthermore, individual neurons with distinct task-related firing rate profiles were differently co-modulated with beta amplitude. Phase locking of spikes of individual neurons to LFP oscillations was significant only for the beta range, increased with beta amplitude and was rather independent of the neuron's task-related properties. When two beta bands were simultaneously present in the LFP, half of locked neurons locked to both bands within the same task epoch. In the literature it is often simplistically stated that sensorimotor beta 'de-synchronization' reflects motor cortical activation. However, we find no stereotypical relationship between beta amplitude and the level of single or multi-unit activation, beyond co-modulations with behavior. Beta oscillation amplitude rather trivially reflects the level of rhythmic synchrony across neurons, relatively independent of the firing rate and task selectivity of individual, locked neurons. Moreover, our results may reconcile the disparate roles proposed for beta oscillations in cognition and motor control, demonstrating distinct and sometimes co-active beta bands, with contrasting modulations in relation to event expectancy and motor maintenance.

Disclosures: **B.E. Kilavik:** None. **A. Riehle:** None.

Poster

710. Motor Learning: Mechanisms

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Topic: D.05. Visual Sensory-motor Processing

Support: Friends of the Waisman Center

Title: Unimanual and bimanual reach to grasp movements in typically developing children and children with Autism Spectrum Disorder

Authors: R. RODGERS, G. BELLINGER, C. FREER, B. TRAVERS, *A. H. MASON; Kinesiology, Univ. of Wisconsin - Madison, Madison, WI

Abstract: Autism Spectrum Disorders (ASDs) are characterized by impairments in social communication and repetitive behaviors and/or restricted interests (APA, 2014). Differences in movement planning, preparation, and execution have been found in children with ASD compared to typically developing (TD) children (Mari, 2003). We examined coordination using unimanual and bimanual reach to grasp tasks and induced an unexpected target perturbation during ongoing bimanual movements. Fifteen children and adolescents with ASD (10.6 ± 2.5 years) and 15 children with TD (10.1 ± 2.6 years) participated in the study. Participants with ASD had a previous diagnosis of autism and met cutoffs on both the Social Communication Questionnaire and the Autism Diagnostic Observation Scale. In unimanual conditions participants were asked to reach out with the right or left hands to grasp and lift targets located at near (18 cm) or far (28 cm) distances. During bimanual conditions participants grasped two targets located at the same distance or at different distances. On 42% of bimanual trials, a perturbation occurred immediately after movement onset. Either one or both of the targets located at the far distance were extinguished and new targets were illuminated at the near distance. Participants performed a total of 88 trials. Kinematic data were obtained for the index finger, thumb and wrist of both hands using a VisualEyez (Phoenix Technologies) 3D motion capture system. Analysis of movement times in the unimanual conditions revealed a significant main effect of Group ($F_{1,28}$=3.98, p=.056) . Children with ASD were slower (1050.1 ± 64 ms) than typically developing children (869.5 ± 64 ms). A Hand X Group interaction was also found ($F_{1,28}$=4.4, p=.046) which indicated that typically developing children were significantly slower when reaching with their left hands (Left = 916 ± 66; right = 822 ± 63 ms), whereas children with ASD used the same movement time regardless of hand (Left = 1062 ± 66; Right = 1037 ± 63 ms). Main effects of condition were found for movement times in the unperturbed ($F_{1,84}$=10.6, p<.01) and perturbed ($F_{2,56}$=3.3,p=.04) bimanual trials. Children were faster when both objects were initially positioned at, or perturbed to, the near locations than when one or both objects were at the far location. Surprisingly, no main effects or interactions of Group were found for movement time in the bimanual conditions. These results may suggest that deficits in performance in children with ASD are task specific and will be interpreted with respect to coupling between the limbs in bimanual performance. Further analyses will also probe more detailed kinematic parameters for evidence of Group differences.

Disclosures: **R. Rodgers:** None. **G. Bellinger:** None. **C. Freer:** None. **B. Travers:** None. **A.H. Mason:** None.

Poster

710. Motor Learning: Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 710.25/V24

Topic: D.05. Visual Sensory-motor Processing

Support: DFG grant Fi 1567/4-1

Title: Decoding movement goals from human reach-related areas in a pro-/anti-reach task

Authors: ***H. GERTZ**[1], A. LINGNAU[2], K. FIEHLER[1];
[1]Justus-Liebig Univ. Giessen, Exptl. Psychology, Giessen, Germany; [2]Ctr. for Mind/ Brain Sci., Trento, Italy

Abstract: In a recent human fMRI study we investigated movement planning in a delayed pro-/anti-reach task (Gertz & Fiehler, 2015). Participants planned and performed right arm reaches towards the position of a previously presented visual target (condition Pro) or to its mirrored location (condition Anti). In condition Pro, the position of the visual target and the movement goal thus coincided, while condition Anti allowed for dissociating the position of the visual target from the position of the movement goal. Visual targets were presented either in the left or right visual field while participants fixated at the center of the screen. Using univariate GLM analyses we found activation in areas related to reach planning in the dorsal premotor cortex, the anterior intraparietal sulcus and the superior parietal cortex (SPL) in both the Pro and Anti condition. Within this reaching network we showed that the left SPL, contralateral to the moving arm, encodes the inferred movement goal rather than the physically present visual target during reach planning. Here we used linear discriminant analysis (LDA)-based multivariate pattern (MVP) analysis to test whether we can predict the position of the upcoming movement goal and/or the position of the previously presented visual target within areas of the reaching network. To this end, we trained the classifier on conditions with the same movement goal, i.e. [condition Pro/left visual targets + condition Anti/right visual targets] and [condition Pro/right visual targets + condition Anti/left visual targets], and on conditions with the same visual target, i.e. [condition Pro/left visual targets + condition Anti/left visual targets] and [condition Pro/right visual targets + condition Anti/right visual targets]. We observed significant movement goal decoding in the SPL and in the anterior intraparietal sulcus. These results confirm our previous findings based on

whole brain GLM analyses and suggest that the movement goal rather than the visual target is encoded in human posterior parietal areas in a pro-/anti-reach task.

Disclosures: H. Gertz: None. **A. Lingnau:** None. **K. Fiehler:** None.

Poster

710. Motor Learning: Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 710.26/V25

Topic: D.05. Visual Sensory-motor Processing

Support: CIHR Grant MOP125915

Title: Comparison of cognitive motor integration deficits associated with concussion and Alzheimer's disease risk

Authors: A. T. VAN WIJNGAARDEN[1], M. DALECKI[1], K. M. HAWKINS[1], *L. E. SERGIO[1,2];
[1]Sch. Kinesiol & Hlth. Sci., York Univ., Toronto, ON, Canada; [2]Ctr. for Vision Res., Toronto, ON, Canada

Abstract: We compared cognitive-motor integration between individuals with a history of concussion versus those with increased Alzheimer's disease risk (through a diagnosis of mild cognitive impairment or a familial history of dementia). Our previous research has shown performance decrements with concussion and early-stage Alzheimer's disease patients when completing visually guided movements that rely on visual-spatial and rule-based transformations. Here we examined the ways in which performance of individuals affected by concussion versus Alzheimer's disease risk differed, accounting for age-related psychomotor changes. Children with history of concussion (n = 22; mean age 13.2 ± 1.4 years) and adults with increased Alzheimer's disease risk (n = 22, mean age 67.5 ± 11.4 years) made finger movements on a touch-screen in two separate spatial planes to displace a cursor from a central to peripheral target. In the standard condition, the finger slid over the viewed horizontal surface directly to the target. In the non-standard "decoupled" condition, targets were viewed on a vertical monitor, spatially dissociated from horizontal-plane finger motion. Also, visual feedback was reversed such that the direction of cursor movement was rotated 180° from that of finger movement. Upon task decoupling, adults at increased Alzheimer's disease risk were less efficient at making movement corrections, produced less direct path trajectories with reduced accuracy, but were better at controlling ballistic path length to correct for errors versus those in the concussion

group. Both groups experienced similar increases in reaction time. Finally, individuals with a history of concussion had more difficulty executing voluntary movements with precision when the visual input and motion were spatially incongruent. A discriminant analysis correctly differentiated individuals with a history of concussion from those at increased Alzheimer's disease risk with 91% accuracy, based on task performance. These data suggest that the brain networks responsible for cognitive-motor integration are differently affected by concussion and Alzheimer's disease risk. Cognitive-motor integration provides a sensitive means to detect long-term functional impairment in a way that can distinguish mild traumatic brain injury from dementia risk, and may serve as an improved means of monitoring Alzheimer's disease-related functional decline.

Disclosures: A.T. van Wijngaarden: None. **M. Dalecki:** None. **K.M. Hawkins:** None. **L.E. Sergio:** None.

Poster

710. Motor Learning: Mechanisms

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Program#/Poster#: 710.27/V26

Topic: D.05. Visual Sensory-motor Processing

Support: CIHR Grant (125 915)

Title: Children show cognitive-motor integration deficits nearly two years after concussion

Authors: *M. DALECKI[1], D. ALBINES[1], A. MACPHERSON[2], L. E. SERGIO[3];
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Abstract: In a previous study we observed cognitive-motor integration (CMI) deficits in asymptomatic adult athletes with a history of concussion, especially when the brain had to decouple vision from action. In the present study, we examine whether the same is true for children. Asymptomatic children with a history of concussion (n=50, 0.25-48 months post, mean 12.87; mean age 12.84 yr) and age-matched controls (n=49, mean age 11.63 yr) performed two tasks. They had to slide a cursor from a central to a peripheral target using their finger, using a dual-touch screen laptop. Participants performed one direct-interaction task where target location and motor action were aligned, and a CMI task where targets were in a different plane from hand motion, and visual feedback was reversed (i.e. decoupling between vision and action). We

analyzed multiple movement timing (e.g. reaction time, movement time) and trajectory (e.g. pathlength, reaching precision) parameter. We observed a significant impairment in both movement timing and trajectory formation for children with concussion history. Specifically, we found a main effect of group and condition on both timing and movement accuracy/precision measures. Importantly, we observed an interaction of concussion history and level of required CMI on a number of variables. We also observed a significant relationship between the length of time since concussion and movement timing in the CMI condition. Notably, not until nearly two years later did children with concussion history perform the CMI task at the same baseline level as children with no concussion history. We suggest that these performance deficits are due to concussion-induced disruptions in the fronto-parietal networks responsible for rule-based movement guidance. We previously observed only timing but not trajectory deficits in varsity athletes with concussion history, hence these networks may be more vulnerable in the developing brain. The observed prolonged deficits in CMI further suggest that current return to sport/school/work assessments that test thinking and movement separately, something crucial in sport and daily life, are not fully capturing functional abilities post-concussion. Moreover, the findings of the present study might also be important for a better understanding of negative long-term effects that can appear following concussion, and help explain why seemingly asymptomatic athletes are more vulnerable to further brain injury.

Disclosures: **M. Dalecki:** None. **D. Albines:** None. **A. Macpherson:** None. **L.E. Sergio:** None.

Poster

710. Motor Learning: Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 710.28/V27

Topic: D.17. Voluntary Movements

Support: Minnesota's Discovery, Research, Innovation Economy (MnDRIVE) Initiative Graduate Fellowship

Title: Primed vs unprimed paired associative stimulation: A single-subject proof of principle exploration

Authors: ***K. FROST**, M. CHEN, J. CAREY;
Univ. of Minnesota, Minneapolis, MN

Abstract: Background/Purpose: Neuroplasticity governs mechanisms of cortical reorganization, adaptation and recovery following neural injury. Paired associative stimulation (PAS) induces a

long-lasting change in neuroplasticity by pairing a peripheral nerve stimulus with a cortical stimulus. However, preceding PAS treatment intended to induce neuroplastic change in one direction with a treatment intended to weight synaptic plasticity in the opposite direction may deploy homeostatic synaptic mechanisms that prime the motor cortex to better respond to the second treatment, resulting in a larger and more consistent change in cortical excitability. Exploring principles of homeostatic synaptic plasticity, this study aims to provide proof of principle for primed PAS to augment neuromodulation. Subject: Single healthy human (male, age 65). Methods: We applied five different PAS conditions 1) control, 2) N20+5ms unprimed, 3) N20-5ms unprimed, 4) N20+5ms priming followed by N20-5ms conditioning and 5) N20-5ms priming followed by N20+5ms conditioning in this subject with one-week washout periods between sessions. The N20 was determined using electroencephalography. Change in corticospinal excitability was assessed by comparing the peak-to-peak average of 30 motor evoked potentials elicited by single pulse transcranial magnetic stimulation before PAS treatment and 0, 10, 20, 30, 40, 50 and 60 minutes following treatment. Results: Preceding facilitatory PAS with inhibitory PAS significantly increased cortical excitability more than facilitatory PAS alone ($p<.05$). Preceding inhibitory PAS with facilitatory PAS compared to inhibitory PAS alone was equivocal. Conclusions: This study suggests proof of principle that priming of motor cortex, previously demonstrated with other methods of non-invasive brain stimulation to accomplish more robust neuromodulation, may also apply to PAS and invites larger confirmation studies and possible translation to rehabilitation following neural injury.

Disclosures: **K. Frost:** None. **M. Chen:** None. **J. Carey:** None.

Poster

711. Oral Motor and Speech

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 711.01/V28

Topic: D.17. Voluntary Movements

Title: Pre-articulatory activity in human sensory cortex

Authors: ***K. FORSETH**[1], E. BARTOLI[1], G. HICKOK[2], N. TANDON[1];
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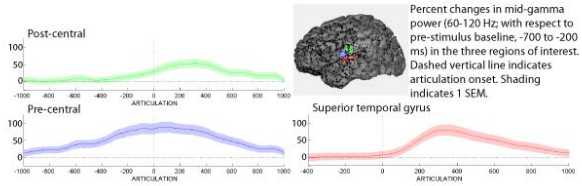
Abstract: During volitional movements, motor plans are previewed in sensory regions, allowing the precise real time auditing of the expected sensory consequences. This process allows for the refinement of the control of movement by comparison between the predicted sensory consequences and the actual ones. Therefore, the comparison between this “efference copy” of

motor commands and the actual sensory feedback is crucial during the control of motor abilities. Speech production is a particularly interesting type of motor skill, since multiple articulators needs to be precisely tuned to accurately produce sounds used to communicate. The sensory-motor interplay during speech production, strongly predicted by the proposed role of the efference copy in motor control, is still lacking categorical neurophysiological evidence. Here, we sought to detect the neural signature of efference copy related to articulation using a simple word production task. To this end, we recorded intracranial EEG (icEEG) in a sample of patients undergoing preoperative assessment of epileptic activity with intracerebral and subdural electrodes. Electrodes in pre-central, post-central and superior temporal gyri were specifically selected for the analysis. icEEG recordings were aligned to articulation onset and power changes related to gamma (60-120 Hz) oscillations were calculated using a time window locked to articulation onset (-1000; -1000 ms). Preliminary analyses show that, somatosensory (post-central) electrodes exhibit significant activity in the gamma band starting at ~200 ms prior the onset of articulation, overlapping in time with motor (pre-central) activation. However activity in the superior temporal gyrus (STG) does not clearly precede articulation onset. This pre-articulatory activation of somato-sensory regions is suggestive of the processing of ongoing motor commands relative to the expected sensory feedback from periphery. The information exchanged between these regions might represent the signature of sensory-motor mapping during articulatory control.



Disclosures: **K. Forseth:** None. **E. Bartoli:** None. **G. Hickok:** None. **N. Tandon:** None.

Poster

711. Oral Motor and Speech

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 711.02/V29

Topic: D.17. Voluntary Movements

Support: NIH Grant R01 DC007603

CIHR Grant MOP-137001

Title: Motor planning for speech modulates auditory responses differently in stuttering vs. nonstuttering adults

Authors: A. DALIRI[1], *L. MAX[2];
[1]Boston Univ., Boston, MA; [2]Univ. Washington, Seattle, WA

Abstract: Sensorimotor integration involves a predictive priming of task-relevant sensory systems. Here, we investigated modulation of the auditory system during speech movement planning in adults with typical speech and adults who stutter. In Study 1, we recorded auditory evoked potentials (AEPs) in response to probe tones presented during speech movement planning in a speaking condition and control conditions without movement planning. Nonstuttering subjects showed a modulation of the auditory system (reduced N1 amplitude in the speaking condition) that was not observed for the stuttering group. These data suggest that speech movement planning involves anticipatory adjustments in the auditory feedback pathway, and that stuttering is associated with deficiencies in this modulation process. In Study 2, we investigated whether modulation of the auditory system occurs only when making predictions during movement planning. We recorded AEPs in response to probe tones delivered (a) while subjects anticipated hearing their self-produced speech (speaking condition), (b) while subjects anticipated hearing a played-back version of their pre-recorded speech (hearing condition), and (c) during a control condition without auditory input (silent reading condition). For the nonstuttering group, there was modulation of the N1 amplitude in both the speaking and hearing conditions vs. the silent reading condition. For the stuttering group, there was no N1 modulation in either condition with auditory input. Thus, stuttering individuals have difficulties with making auditory predictions in general. In Study 3, we investigated whether pre-speech auditory modulation relates to the extent of reliance on auditory feedback during speech production. Reliance on auditory feedback was estimated with a sensorimotor adaptation paradigm. Auditory modulation was examined by presenting probe tones (a) prior to speaking with normal auditory feedback (NAF), (b) prior to speaking with delayed auditory feedback (DAF), and (c) in a control condition (silent reading). Adaptation was limited for stuttering vs. nonstuttering subjects, and auditory modulation was again nonsignificant in the stuttering group. Interestingly, DAF tended to reduce auditory modulation for nonstuttering subjects but increase it for stuttering subjects. Overall, auditory-motor adaptation correlated with the extent of change in auditory modulation from NAF to DAF. Hence, auditory modulation during speech planning relates to the extent of reliance on auditory feedback during speech production, and stuttering individuals show less auditory modulation and a reduced reliance on auditory feedback.

Disclosures: A. Daliri: None. **L. Max:** None.

Poster

711. Oral Motor and Speech

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01DC012502

NSERC-Canada

FQRNT-Quebec

Title: Perceptual context effects on auditory feedback processing in speech production

Authors: *N. BOURGUIGNON[1,2,3], S. R. BAUM[4,3], D. M. SHILLER[2,1,3];
[1]CHU Sainte-Justine, Montreal, QC, Canada; [2]École d'orthophonie et d'audiologie, Univ. de Montréal, Montreal, QC, Canada; [3]Ctr. for Res. on the Brain, Language and Music, Montreal, QC, Canada; [4]Sch. of Communication Sci. and Disorders, McGill Univ., Montreal, QC, Canada

Abstract: We investigated the effects of changes in formant structure of externally presented speech signals on participants' auditory perception of their own speech output during a word production task. The study involved a novel combination of two previously established research paradigms. The first involves sensorimotor adaptation to altered auditory feedback (AAF) during speech production. Evidence from this paradigm typically supports the notion of speech sounds as accurate, stable sensory targets that serve as the primary goals of speech movements. The second relates to extrinsic talker normalization of vowel perception through the presentation of carrier-phrases spoken with different formant patterns. In contrast to AAF, results from this paradigm illustrate the adaptive plasticity of speech sensory targets to external speech variability. These combined paradigms allowed us to understand the extent to which formant frequencies of a carrier-phrase presented immediately prior to word production could also serve as a frame of reference for the perception of self-generated speech outcomes, thereby influencing subsequent speech production targets. Subjects read aloud single words containing the vowel /Ɛ/ (e.g., "bet", "head") under conditions of normal or altered auditory feedback. Real-time feedback alteration involved a decrease in F1 frequency, resulting in a vowel perceived to be closer to /I/ (e.g., "bit", "hid"). Consistent with previous research, we expected this perturbation to yield opposite compensatory responses in subjects' output. Before each production, subjects heard a phrase spoken with one of three different formant patterns, simulating differences in vocal tract properties of three different talkers (nearly identical to carrier phrases previously shown to induce changes in the perception of an ambiguous vowel between /Ɛ/ and /I/). We predicted that

if the carrier phrase influenced subjects' perception of their own vowel formants during word production, the resulting change would impact the degree of motor adaptation to their F1-altered auditory feedback. Our results suggest that the formant frequencies of a carrier-phrase presented immediately prior to word production serve as a frame of reference for the perception of self-generated speech, thereby influencing subsequent speech targets. This finding extends recent evidence indicating that the auditory processing of speech sounds for speech production is highly adaptive under a range of different conditions. Altogether, this evidence has important implications in the modeling of speech sensory targets as stable, accurate representations at the core of speech movements.

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Poster

711. Oral Motor and Speech

Location: Hall A

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Program#/Poster#: 711.04/V31

Topic: D.17. Voluntary Movements

Support: NIH R01DC007658

Title: Asymmetrical specialization of cortical-striatal white matter connections for motor speech control

Authors: *J. J. SIDTIS[1], A. MUBEEN[1], B. ARDEKANI[2], D. SIDTIS[3];
[1]Geriatrics, [2]Ctr. for Biomed. Imaging and Neuromodulation, Nathan Kline Inst., Orangeburg, NY; [3]New York Univ., New York, NY

Abstract: The specialization of the left cerebral hemisphere for speech and language became a cornerstone of functional localization in the brain following the observations of Broca and Wernicke, but interest in the effects of focal brain damage waned with the growth of functional imaging. However, brain maps derived from imaging have not always been consistent with lesion studies. Specifically, the strong left-hemisphere lateralization of speech and language has not been reflected in the bilaterality of brain activation in functional imaging. We have previously shown that despite bilateral activation during speech tasks, performance can be predicted by a combination of an increase in blood flow in the left inferior frontal region and decrease in the right caudate. In the present study, we examined the structural connections between the inferior frontal regions and the caudate nuclei using diffusion tensor imaging in normal volunteer subjects (n = 25) and assessed the relationships between the measures of white

matter connections and speech characteristics obtained from acoustic analysis of speech samples obtained at a separate evaluation. Probabilistic tractography was used to estimate the strength of the connections between the head of the caudate nucleus (the seed) and the inferior frontal region (the target), both ipsilaterally and contralaterally. After the probabilistic connections were established for each subject, the fractional anisotrophy (FA) was measured for these tracts. The probabilistic tractography demonstrated that the majority of fiber connections were ipsilateral, but contralateral connections were present as well. The inter-subject variability in the relative number of connections between the right caudate and the left inferior frontal region was significantly associated with the inter-subject variability in the acoustic measures of stability for frequency (Spearman's coefficients for the repetition of the syllables /pa/ = 0.46; /ta/ = 0.5; /ka/ = 0.46; /pataka/ = 0.52) and amplitude (Spearman's coefficients for the repetition of the syllables /pa/ = 0.43; /ta/ = 0.5; /ka/ = 0.49; /pataka/ = 0.49) in the speech signals, echoing the significant relationship in blood flow in these regions during speech. A similar pattern was found for FA. No other cortical-striatal connections demonstrated these relationships with speech characteristics. These results suggest that white matter connections may share some degree of functional specialization with the structures that they connect.

Disclosures: **J.J. Sidtis:** None. **A. Mubeen:** None. **B. Ardekani:** None. **D. Sidtis:** None.

Poster

711. Oral Motor and Speech

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Program#/Poster#: 711.05/V32

Topic: D.17. Voluntary Movements

Support: Royal Society Grant RG130041

ESRC Grant ES/L01257X/1

Title: Voluntary imitation of fundamental frequency and vocal tract length in human speech: A multimodal investigation using functional and real-time anatomical MRI

Authors: *C. MCGETTIGAN[1,2], D. CAREY[1], V. CARTEI[3], M. MIQUEL[4];
[1]Royal Holloway, Univ. of London, Egham, United Kingdom; [2]Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom; [3]Dept. of Psychology, Univ. of Sussex, Brighton, United Kingdom; [4]Queen Mary Univ. of London, London, United Kingdom

Abstract: The human voice is a highly flexible channel for the expression of linguistic, emotional and social information. Perceptually, fundamental frequency (F0; closely related to pitch) and formant spacing (an index of vocal tract length; VTL) are important cues for the extraction of indexical characteristics such as sex and individual identities. Behavioural research has further shown that talkers instinctively modulate these cues to emulate various physical and social attributes (e.g. masculinity/femininity: Cartei et al., 2012; attractiveness: Hughes et al., 2014). The current study forms the first combined acoustic, articulatory and neurobiological investigation of these paralinguistic aspects of vocal behavior. Recordings of monosyllables (e.g. "bead") from individual participants were used to create synthethic arrays of target voices varying in F0 and VTL along two axes: one biologically typical axis ranging from voices with lower F0s and longer VTLs to those with higher F0s and shorter VTLs (i.e. from adult males toward adult females and children), and one less typical axis ranging from voices with lower F0s and shorter VTLs to those with higher F0s and longer VTLs. Acoustic analyses of imitations from 22 adults (11 M) showed that talkers reproduced significant changes in F0 and VTL along both axes, but that F0 was better differentiated than VTL (particularly for atypical targets; Figure 1a). In a second study, real-time anatomical MRI (rtMRI) of the vocal tract allowed us to measure the dynamics (at 8 fps) of VTL modulation as well as the functional correlates of perception and imitation using fMRI. The rtMRI data showed voluntary raising and lowering of the larynx to imitate varying VTLs (Figure 1b) - in-scanner rtMRI and acoustic recordings were then used to probe individual differences in the functional correlates of voluntary vocal imitation within auditory and somatomotor networks of the brain.

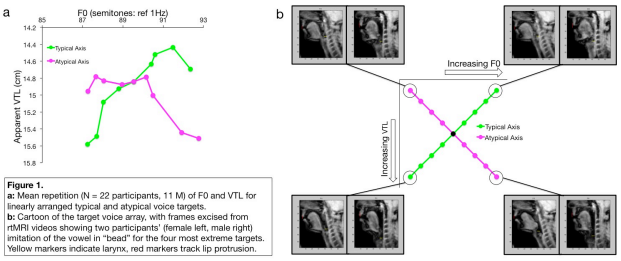

Figure 1.
a: Mean repetition (N = 22 participants, 11 M) of F0 and VTL for linearly arranged typical and atypical voice targets.
b: Cartoon of the target voice array, with frames excised from rtMRI videos showing two participants' (female left, male right) imitation of the vowel in "bead" for the four most extreme targets. Yellow markers indicate larynx, red markers track lip protrusion.

Disclosures: **C. McGettigan:** None. **D. Carey:** None. **V. Cartei:** None. **M. Miquel:** None.

Poster

711. Oral Motor and Speech

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 711.06/V33

Topic: D.17. Voluntary Movements

Support: NIH Grant DC014211-02

Title: Disrupted feedforward but spared feedback control during speech in patients with cerebellar degeneration

Authors: *B. PARRELL[1], J. HOUDE[2], S. NAGARAJAN[3], R. IVRY[1];
[1]Dept. of Psychology, Univ. of California, Berkeley, Berkeley, CA; [2]Dept. of Otolaryngology, [3]Dept. of Radiology and Biomed. Imaging, Univ. of California, San Francisco, San Francisco, CA

Abstract: People with ataxia due to cerebellar degeneration exhibit a range of speech deficits referred to as ataxic dysarthria (AD). Symptoms include slow speech, excessively equal syllable durations, and (paradoxically) increased durational variability in syllable repetition tasks. These deficits reflect an inability to accurately plan the fast, precise movements of the speech articulators necessary for fluent speech—computations that are highly dependent on a feedforward control system. This impairment may cause people with AD to become overly reliant on feedback control when speaking, leading to temporal delays and instability inherent in any closed-loop feedback control system. These hypotheses are consistent with results from other motor domains (reaching, walking) that suggest the cerebellum is crucial to accurate feedforward control, with reduced involvement in feedback control. We compared feedforward and feedback control in two altered auditory feedback studies. Such studies—analogous to the methods used to study sensorimotor adaptation in reaching—involve real-time manipulation of the speech formants, with the end result that speakers hear a different vowel than that they actually produced (either slightly altered or categorically different, depending on the manipulation). Healthy speakers compensate for these manipulations, adapting the feedforward system across productions to consistent alterations and compensating within a vowel for unexpected perturbations via the feedback system. We tested the feedforward control system by measuring adaptation to consistent vowel perturbations, introduced gradually in one block of trials and abruptly in another. For dependent variables, we measured produced formants in words spoken during 1) maximum adaptation at the end of the perturbation phase and 2) probe trials in which the production was accompanied by loud noise to mask auditory feedback. People with AD adapted less than age-matched healthy controls in both measures to both the gradual and abrupt perturbations. To test the integrity of the feedback control system, we examined the response of the participants to unexpected vowel formant perturbations. In this case, people with AD were unimpaired in their compensatory behavior relative to control speakers. Combined, these results support the hypothesis that cerebellar degeneration selectively disrupts feedforward speech motor control.

Disclosures: **B. Parrell:** None. **J. Houde:** None. **S. Nagarajan:** None. **R. Ivry:** None.

Poster

711. Oral Motor and Speech

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 711.07/V34

Topic: D.17. Voluntary Movements

Support: Northwestern Memorial Foundation Dixon Translational Research Grant

Title: Cortical representation of articulatory gestures and phonemes during speech production

Authors: *E. M. MUGLER[1], M. GOLDRICK[2], R. D. FLINT[1], M. C. TATE[3], J. M. ROSENOW[3], M. W. SLUTZKY[1];
[1]Neurol., Northwestern Univ., Chicago, IL; [2]Linguistics, Northwestern Univ., Evanston, IL; [3]Neurosurg., Northwestern Univ., Chicago, IL

Abstract: Brain-machine interfaces (BMI) that decode speech could greatly improve the quality of life for people who are completely paralyzed, or "locked-in," due to a stroke or ALS, by restoring their ability to communicate. Despite recent successes in decoding certain aspects of speech production from cortical activity, the exact encoding of activity in motor and pre-motor cortices during speech production is unclear. Previous theoretical, acoustic, and fMRI work has suggested multiple levels of sound representation support speech production, from phonemes (whole speech sounds) to articulatory gestures (movements of individual articulators). A more complete, electrophysiological understanding of the cortical representation of these different levels of speech would aid BMI design. Using electrocorticography (ECoG), we evaluated the degree to which articulatory gestures and phonemes are functionally represented in speech motor and premotor cortices. We recorded ECoG in three subjects with brain tumors who required awake craniotomies to map speech and motor function. Electrode arrays (8x8, Integra Inc., 2.3 mm platinum electrodes, 4 mm inter-electrode spacing) were placed over the ventral motor (M1v), ventral premotor (PMv) and inferior frontal gyri. We sampled cortical activity at 2 kHz, after bandpass filtering from 0.3 to 500 Hz (Blackrock Microsystems). Concurrently, subjects read aloud single consonant-vowel-consonant (CVC) words presented on a monitor at a rate of 1 word every 2 seconds. We sampled audio at 48 kHz using a unidirectional lapel condenser microphone (Califone) and synchronized it with the cortical signal using BCI2000 software. We manually labelled the onset of each phoneme (speech sound). We obtained high-gamma power from ECoG by common-average referencing, bandpass filtering from 70-300 Hz, and applying a Hilbert transform. We then z-scored the high gamma bandpower for each ECoG channel. We separately decoded gestures and phonemes from the band power 200 ms before and after phoneme or gesture onset using linear discriminant analysis (LDA). By separately performing LDA using electrodes covering different anatomical areas, we could analyze changes in speech

representation in different areas of cortex. Gestures were better represented in the primary sensorimotor cortices, while phonemes were better represented in more anterior cortices.

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Poster

711. Oral Motor and Speech

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 711.08/V35

Topic: D.17. Voluntary Movements

Support: FQRNT PDF

Title: Cerebellar tdcs during speech perceptual learning dissociates the act of indicating perception from perceptual change

Authors: *D. R. LAMETTI, L. OOSTWOUD WIJDENES, J. BONAIUTO, S. BESTMANN, J. C. ROTHWELL;
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Abstract: Speech perception is remarkably malleable yet there have been few causal investigations of the brain regions involved in speech perceptual learning. Recent neuroimaging studies suggest that the cerebellum might play a role in perceptual learning. Here we test this idea using transcranial direct current stimulation (tDCS) in combination with a speech perceptual learning task. In the experiments, participants experienced a series of speech perceptual tests designed to measure and then manipulate their perception of the phonetic contrast between the words “head” and “had”. One group received cerebellar tDCS during speech perceptual learning and a different group received “sham” tDCS during the same task. Learning-related changes in speech perception were observed a week after training and the perceptual change transferred to a different phonetic contrast. Cerebellar tDCS did not have a significant impact on the rate of perceptual change or its subsequent retention and transfer. However, for both trained and untrained speech sounds, cerebellar tDCS significantly increased the time it took participants to indicate their perceptual decision with a keyboard press. Using the drift diffusion model, we provide evidence that cerebellar tDCS disrupted processes related to the act of indicating perception while sparing processes related to perceptual decision making. The results suggest that the cerebellum’s role in speech is restricted to the motor domain.

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Poster

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Topic: D.17. Voluntary Movements

Support: American Speech-Language-Hearing Association (ASHA) Advancing Academic-Research Careers (AARC) Award

Title: Testing a new localizer task for the central control of vocalization using variations in voice onset for words

Authors: ***M. DIETRICH**[1], E. J. HUNTER[2], S. H. FREY[3];
[1]Communication Sci. and Disorders, Univ. of Missouri, Columbia, MO; [2]Michigan State Univ., East Lansing, MI; [3]Washington Univ., St. Louis, MO

Abstract: The study of the cortical control of vocalization for speech in humans has been facilitated by the development of event-related sparse-sampling fMRI experimental designs. We now have basic knowledge on the localization of the laryngeal motor cortex and primary and secondary vocal areas. However, knowledge on networks in the brain subserving vocalization under various task conditions remains scarce. Foundational studies on the central of vocalization focused on the production of isolated sounds and syllables without semantic meaning, that is, simple voluntary laryngeal motor behaviors (e.g., glottal stops). Learning more about the control of voice during speech, that is, complex voluntary laryngeal motor behaviors, will help to better understand functional voice disorders. Functional voice disorders, also known as primary muscle tension dysphonias, are characterized by excessive or disorganized activity of the intrinsic or extrinsic laryngeal muscles. Patients typically perceive increased vocal effort or strain. The objective of the current study is to test a new localizer task for complex laryngeal motor behavior. To that end, we focus on voice production at the word level. Twelve vocally healthy participants produce vowel-initial words in two manners: breathy vs. hard glottal onset. The manners differ in vocal fold adduction (closure) with greater vocal fold engagement for the hard glottal onset. Words chosen from the Psycholinguistic Database are monosyllabic, start with a vowel, and exclude verbs. Our word list shows a significant difference in mean airflow during voicing for words produced with a breathy vs. hard glottal onset (0.31 L/s vs. 0.18 L/s), which validates the differential vocal fold engagement. We predict that the subtraction of fMRI BOLD

activations will localize laryngeal motor cortical areas that are relevant for speech production (ventromedial area BA4p and dorsolateral area BA6) and that are viable as functional ROIs. We will also measure each participant's airflow for voicing and laryngeal airway resistance (subglottal air pressure divided by mean airflow for voicing). In the future, the new localizer task will be used with participants who report vocal effort and strain. Greater laryngeal airway resistance is often perceived as vocal strain. We predict that hard glottal onset will be characterized by greater activity in the laryngeal motor cortex, premotor cortex, and cerebellum (CB-VI, VIIIA) compared with a breathy voice onset. The overall goal of our research is to determine the pattern of brain activation underlying increased vocal effort to better understand individual differences in risk for voice disorders.

Disclosures: M. Dietrich: A. Employment/Salary (full or part-time):; University of Missouri. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); American Speech-Language-Hearing Association (ASHA) Advancing Academic-Research Careers (AARC) Award. **E.J. Hunter:** A. Employment/Salary (full or part-time):; Michigan State University. **S.H. Frey:** A. Employment/Salary (full or part-time):; Washington University.

Poster

711. Oral Motor and Speech

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 711.10/V37

Topic: D.17. Voluntary Movements

Title: Speech consists of simultaneous activation of the emotional and somatic motor system

Authors: *G. HOLSTEGE[1], H. H. SUBRAMANIAN[2];
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Abstract: In animals and humans vocalization is generated by the emotional motor system, in which the mesencephalic periaqueductal gray (PAG) as well as the nucleus retroambiguus (NRA) play a central role. The caudal PAG has direct and strong access to the NRA, located in the caudal medullary ventrolateral tegmentum. Although the NRA is not mentioned in several recent review papers concerning vocalization and speech, it is the only cell group in the central nervous system that has direct and specific access to not only the motoneurons of pharynx and larynx, but also to the motoneurons innervating diaphragm, intercostal, abdominal and pelvic floor muscles.1 Together these muscles determine intra-abdominal-pelvic, intra-thoracic and subglottic pressure, necessary for generating vocalization. Lesions involving the caudal PAG

have been shown to result in mutism in animals as well as in humans. Only humans have the ability to modulate vocalization into words and sentences, resulting in speech. The descending pathways producing this modulation take part in the lateral component of the somatic or voluntary motor system, which in humans primarily originates in the primary motor cortex. These motor corticobulbar fibers have access to the motoneurons innervating face, mouth, tongue, larynx and pharynx and their premotor interneurons in the caudal pontine and medullary lateral tegmental field, but not to the nucleus retroambiguus.2 In order to generate speech, humans produce vocalization by activating the connection from the orbitofrontal cortex to the PAG-NRA-motoneuronal pathway, and modulate this vocalization by activating the primary motor cortex-motoneuronal pathway. It explains why patients with lesions in Broca's area (speech memory neurons in Brodmann's areas 44 and 45) are no longer able to produce speech (motor aphasia), but still can vocalize, while patients with lesions in the caudal PAG are mute. References 1 Holstege, G. (1989) An anatomical study of the final common pathway for vocalization in the cat. J. Comp. Neurol. 284: 242-252 2 Kuypers, H.G.J.M. (1958) Corticobulbar connections to the pons and lower brain-stem in man: an anatomical study. Brain 81: 364-388

Disclosures: G. Holstege: None. **H.H. Subramanian:** None.

Poster

711. Oral Motor and Speech

Location: Hall A

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Topic: D.17. Voluntary Movements

Support: ERC Speech Units

Title: Adaptation to altered auditory feedback in speech to assess transfer of learning in complex serial movements

Authors: T. CAUDRELIER[1], J.-L. SCHWARTZ[1], P. PERRIER[1], C. SAVARIAUX[1], *A. ROCHET-CAPELLAN[2];
[1]Univ. Grenoble Alpes, Gipsa-Lab, CNRS, Saint-Martin d'Hères, France; [2]DPC, Gipsa-Lab, Saint-Martin d'Hères, France

Abstract: Using bird song as a model to understand generalization in motor learning, Hoffman and Sober recently found that adaptation to pitch-shift of birds' vocal output transfered to the production of the same sounds embedded in a different serial context (J. Neurosc 2014). In

humans, speech learning has been found to transfer as a function of the acoustical similarity between the training and the testing utterances (Cai et al. 2010, Rochet-Capellan et al. 2011) but it is unclear if transfer of learning is sensitive to serial order. We investigate the effects of serial order on transfer of speech motor learning using non-words sequences of CV syllables. Three groups of native speakers of French were trained to produce the syllable /be/ repetitively while their auditory feedback was altered in real time toward /ba/. They were then tested for transfer toward /be/ (control), /bepe/ or /pebe/ under normal feedback conditions. The training utterance was then produced again to test for after-effects. The auditory shift was achieved in real time using Audapter software (Cai et al. 2008). Adaptation and transfer effects were quantified in terms of changes in formants frequencies of the vowel /e/, as a function of its position and the preceding consonant in the utterance. Changes in formant frequencies in a direction opposite to the shift were significant for ~80% of the participants. Adaptation was still significant for the three groups in the after-effect block. Transfer effects in the /bepe/ and /pebe/ groups were globally smaller than that of the control group, particularly when the vowel /e/ came after /p/ and/or was in second position in the utterance. Taken together, the results suggest that transfer of speech motor learning is not homogenous and as observed by Hoffman and Sober, depends on the serial context of a sound within the utterance. Cai S, Boucek M, Ghosh SS, Guenther FH, Perkell JS. (2008). A system for online dynamic perturbation of formant frequencies and results from perturbation of the Mandarin triphthong /iau/. In Proceedings of ISSP, France, 2008. pp. 65 Cai, S., Ghosh, S. S., Guenther, F. H., & Perkell, J. S. (2010). Adaptive auditory feedback control of the production of formant trajectories in the Mandarin triphthong/iau/and its pattern of generalization. The Journal of the Acoustical Society of America, 128(4), 2033-2048. Hoffmann, L. A., & Sober, S. J. (2014). Vocal generalization depends on gesture identity and sequence. The Journal of Neuroscience, 34(16), 5564-5574. Rochet-Capellan, A., Richer, L., & Ostry, D. J. (2012). Nonhomogeneous transfer reveals specificity in speech motor learning. Journal of neurophysiology, 107(6), 1711-17.

Disclosures: **T. Caudrelier:** None. **J. Schwartz:** None. **P. Perrier:** None. **C. Savariaux:** None. **A. Rochet-Capellan:** None.

Poster

711. Oral Motor and Speech

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Program#/Poster#: 711.12/V39

Topic: D.17. Voluntary Movements

Support: NIH Grant R01 NS026413

Title: Vocal motor development and coordination in wild type and Foxp2 heterozygous mutant mice

Authors: *G. A. CASTELLUCCI[1,2], D. A. MCCORMICK[1];
[1]Yale Univ., New Haven, CT; [2]Haskins Labs., New Haven, CT

Abstract: Deleterious mutations of the FOXP2 gene in humans is known to cause severe Developmental Verbal Dyspraxia (DVD; also called Childhood Dyspraxia of Speech). Interestingly, previous research from our lab has demonstrated that male Foxp2 heterozygous knock out (Foxp2 +/-) mice exhibit deficits in their ultrasonic courtship song. Most notably, while the song syllables of wild type (C57Bl/6J) male mice become significantly longer and less spectrally flat (more tone-like) with age, the syllables of their Foxp2 +/- littermates do not exhibit this developmental trajectory and instead are noisier and shorter at all ages tested (from weaning to adulthood, ~P23 to ~P70 respectively). Furthermore, it was also found that Foxp2 +/- mice produce approximately half the number of song syllables per bout (series of syllables produced in sequence separated by longer periods of silence) than their wild type littermates. While these findings show interesting cursory parallels to the articulatory and prosodic symptoms affecting the speech of individuals with DVD, the biological basis for mouse vocalization production - and therefore its relation to human speech - is not well understood. To better qualify mouse vocalization production, we are currently examining and will present the effects of experience on the vocal development trajectory observed in wild type mice in order to establish whether this trajectory is - like speech - dependent on experience. Additionally, simultaneous electromyography recordings from several muscles recruited during vocalization production (e.g. masseter, temporalis, anterior belly of the digastric, medial pterygoid) will be performed to examine the articulatory strategies employed during production and how they compare to those used for speech. By better understanding the mouse vocalization system, its utility as a limited model of human speech and language can be better evaluated.

Disclosures: G.A. Castellucci: None. **D.A. McCormick:** None.

Poster

711. Oral Motor and Speech

Location: Hall A

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Program#/Poster#: 711.13/V40

Topic: D.17. Voluntary Movements

Support: 1R01DC006243

Title: A temporal predictive code for voice motor control: evidence from neurophysiology and vocal behavior

Authors: *R. BEHROOZMAND[1], S. SANGTIAN[1], O. KORZYUKOV[2], C. LARSON[2]; [1]Communication Sci. and Disorders, Univ. of South Carolina, Columbia, SC; [2]Communication Sci. and Disorders, Northwestern Univ., Evanston, IL

Abstract: The predictive coding model suggests that voice motor control is regulated by the output of a comparative process in which the difference (error) between internally-predicted and sensory feedback stimulus is detected and used to correct motor commands during vocal production. Although motor-driven predictions (e.g. efference copies) are important for vocal error correction, predictions about sensory feedback stimuli can also play a key role in subsequent motor behavior during vocal production and control. In the present study, we studied how predictions about timing of feedback pitch perturbation stimuli can modulate vocal and ERP responses during vowel production. The experiment was carried out in six counterbalanced blocks in which a +100 cents pitch shift stimulus perturbed voice auditory feedback in the middle of vocalizing the vowel sound "a". In three blocks, there was a fixed delay (500, 750 or 1000 ms) between voice and stimulus onset (predictable), whereas in the other three blocks, stimulus onset delay was randomized between 500, 750 and 1000 ms (unpredictable). Analysis of behavioral data showed that for unpredictable stimuli, subjects compensated for feedback perturbations by changing their voice pitch in the opposite direction to stimuli. These compensatory vocal responses were initiated at latencies around 80 ms post-stimulus. However, for predictable stimuli, we found that subjects initiated vocal responses at about 20 ms prior to the onset of stimulus and followed the direction of pitch shifts in their voice feedback. Analysis of the ERPs showed that the amplitudes of the N100 and P200 components were significantly reduced in response to predictable compared with unpredictable stimuli. These findings indicate that predictions about temporal features of sensory feedback can modulate subsequent vocal motor behavior. In the context of the predictive coding model, reduction in the amplitude of the ERP components suggests that the brain may internally simulate and respond to predictable stimuli, and therefore, may assign fewer neural resources for processing auditory feedback during vocal production. This notion is corroborated by our findings showing that the vocal responses were initiated prior to the onset of predictable stimuli. These findings provide new insights into the neural mechanisms of vocal production and motor control.

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Poster

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Topic: D.17. Voluntary Movements

Support: NIH/NINDS R01 NS24328 (PLS)

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NIH/NINDS P30 NS076405 (PLS)

Office of Research and Development, Medical Research Service, Department of Veterans' Affairs (PLS)

NIH/NINDS T32 NS086749 (CMC)

Title: A representation of the larynx in macaque monkey M1

Authors: *C. M. CERKEVICH[1,2,3], P. L. STRICK[1,2,3,4,5];
[1]Systems Neurosci. Inst., [2]Neurobio., [3]Ctr. for the Neural Basis of Cognition, [4]Univ. of Pittsburgh Brain Inst., Univ. of Pittsburgh, Pittsburgh, PA; [5]Res. Service, Veterans Affairs Med. Ctr., Pittsburgh, PA

Abstract: Our understanding of the neural circuitry responsible for the cortical control of hand and arm movements has grown substantially in the last 25 years. In contrast, we know surprisingly little about the cortical areas involved in the control of vocalization in non-human primates. We know even less about the adaptations in these circuits that enable some monkeys to communicate using vocalization and to emit species specific calls on a voluntary basis. Some investigators contend that the voluntary control of vocalization is dependent on the development of direct connections from the primary motor cortex (M1) to motoneurons innervating laryngeal muscles. Others believe that vocalization has evolved through the addition of specialized, higher-order regions of cortex, comparable to Broca's area. As a first step toward resolving these questions, we used the retrograde transneuronal transport of rabies virus (RV) to define the cortical areas in the frontal lobe that control the cricothyroid muscle (CT). The CT is an intrinsic laryngeal muscle that is involved in vocalization. We injected 30µl of the N2C strain (9*10^8 pfu) of RV into the CT of two macaque monkeys. We allowed the animals to survive 90 or 115 hours. This time period was long enough to permit retrograde trans-synaptic transport of RV from the muscle through several synaptically connected neurons to the cerebral cortex. We observed RV-infected neurons in cortical layer V at multiple sites in the frontal lobe that are known to be involved in vocalization (Jurgens, 02; 09), including the anterior cingulate cortex and regions lateral to the ventral premotor area. In addition, we found a substantial number of RV-infected neurons in a far lateral region of primary motor cortex (M1) that includes the surface of the precentral gyrus and the anterior bank of the central sulcus. This region falls in the

presumptive face representation of M1 (e.g., McGuinness et al., 80). This observation provides evidence that a region of M1 in the macaque is the sire of descending input to a muscle involved in vocalization.

Disclosures: C.M. Cerkevich: None. **P.L. Strick:** None.

Poster

712. Voluntary Movement and Motor Plasticity

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Topic: D.17. Voluntary Movements

Support: NSERC RGPIN 401890

Title: Aerobic exercise modulates bilateral intracortical and interhemispheric primary motor cortex excitability

Authors: *J. L. NEVA[1], K. E. BROWN[2], C. S. MANG[2], L. A. BOYD[2];
[1]Dept. of Physical Therapy, [2]Physical Therapy, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The potential for aerobic exercise to modulate neural plasticity, behaviour and cognition is becoming widely known. Recently, work from our lab and others have shown that the excitability of the primary motor cortex (M1) is modulated by a single session of aerobic exercise. Specifically, a single session of lower-limb aerobic exercise modulates unilateral M1 intracortical inhibitory and facilitatory circuitry in the lower-limb and upper-limb representations [1, 2] and facilitates methods of inducing cortical plasticity [3-5]. This suggests that aerobic exercise may alter widespread cortical excitability and inhibitory/facilitatory cortical circuits. However, there is a lack of fundamental understanding of the potential widespread modulation of various intracortical and interhemispheric neural mechanisms supporting the effects of aerobic exercise. The purpose of this study was to investigate the effects of a single bout of moderate aerobic exercise on bilateral intracortical and interhemispheric circuitry within M1. Single and paired pulse transcranial magnetic stimulation (TMS) was used to measure short-interval intracortical inhibition (SICI) and facilitation (SICF), intracortical facilitation (ICF), cortical silent period (CSP) and transcallosal inhibition (TCI) bilaterally in the M1 representation of the non-exercised abductor pollicis brevis (APB). Intracortical and interhemispheric circuitry was measured pre and two time-points post (immediately post and 30 min post) aerobic exercise. Moderate intensity aerobic exercise was performed for 20 min at 65-70% of age-predicated

maximum heart rate on a stationary cycle ergometer. Preliminary results suggest that SICF was enhanced predominantly during the early inter-stimulus intervals. Additionally, SICI decreased and CSP duration was shortened. These results suggest a decreased inhibition and increased facilitation in multiple circuits within M1 after a single session of aerobic exercise. Recruitment for this study is ongoing. Finally, further experiments on the modulation of interhemispheric inhibition of related motor areas are underway. [1] Singh et al. (2014) BMC Sports Sci, Med and Rehabil, 6:23. [2] Yamaguchi et al. (2012) Exp Brain Res, 218:401-6. [3] Mang et al. (2014) J Appl Physiol, 117 (11): 1325-36. [4] Singh et al. (2014) Exp Brain Res, 232:3675-85. [5] McDonnell et al. (2013) J Appl Physiol, 114 (9): 1174-82.

Disclosures: **J.L. Neva:** None. **K.E. Brown:** None. **C.S. Mang:** None. **L.A. Boyd:** None.

Poster

712. Voluntary Movement and Motor Plasticity

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.17. Voluntary Movements

Support: NSERC Grant RGPIN 401890

Title: A single bout of aerobic exercise impacts excitability of cerebellar circuits for a non-exercised upper limb muscle

Authors: ***C. MANG**[1], N. J. SNOW[2], J. L. NEVA[1], K. E. BROWN[2], K. L. CAMPBELL[2], L. A. BOYD[2];
[2]Rehabil. Sci., [1]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Background: A single bout of aerobic exercise impacts the excitability and plasticity of motor cortical (M1) circuits for a non-exercised upper limb muscle. These effects include: a decrease in short-interval intracortical inhibition [1], an increase in intracortical facilitation [1], and increased modulatory responses to inhibitory continuous theta burst stimulation [2] and excitatory M1 paired associative stimulation (PAS, 25 ms interstimulus interval [ISI]) [3]. The effects of a single bout of aerobic exercise on motor-related circuitry outside of M1 have been less studied. In this study, we evaluated the effects of a single bout of aerobic exercise on cerebellar circuits for a non-exercised hand muscle by way of two experiments. Methods: In Experiment 1, response to excitatory M1 PAS was evaluated in 32 young healthy individuals on two occasions: once preceded by 20 min of seated rest, and once preceded by 20 min of high-intensity (90% maximal oxygen uptake) cycling interval training. In 16 individuals PAS was

delivered with a 25 ms ISI (PAS25), and in the other 16 participants with a 21 ms ISI (PAS21). In Experiment 2, three individuals returned for evaluation of cerebellar inhibition on M1 (CBI) at ISIs ranging from 3-8 ms before and after 20 min of seated rest and 20 min of high-intensity interval cycling. Recruitment for Experiment 2 is ongoing. Results: In the first experiment, both PAS25 and PAS21 increased M1 excitability when preceded by exercise, but not rest (Condition x Time interaction: p=0.03). A secondary analysis indicated a greater increase in M1 excitability evoked by exercise and PAS25 (61.0%) compared to exercise and PAS21 (14.3%; p=0.03). Preliminary results from Experiment 2 suggest a release of CBI at ISIs of 6 ms and 7 ms, with an average inhibition of 29.1±18.8% prior to exercise, but facilitation by 22.2±26.9% at the same ISIs following exercise (p=0.02). Conclusion: Previous work has indicated that increases in M1 excitability following PAS25, but not PAS21, involve cerebellar circuits [4]. Thus, our finding that aerobic exercise facilitated response to PAS25 to a greater extent than PAS21 suggests that exercise effects on plasticity in M1 are likely dependent, in part, on cerebellar circuitry. Preliminary results from the second experiment provide further evidence for an effect of aerobic exercise on excitability of cerebellar circuits. [1] Singh et al. (2014) BMC Sports Sci, Med and Rehabil, 6:23. [2] McDonnell et al. (2013) J Appl Physiol, 114 (9): 1174-82. [3] Mang et al. (2014) J Appl Physiol, 117 (11): 1325-36. [4] Hamada et al. (2012) J Physiol, 590 (Pt 10): 2365-74.

Disclosures: C. Mang: None. **N.J. Snow:** None. **J.L. Neva:** None. **K.E. Brown:** None. **K.L. Campbell:** None. **L.A. Boyd:** None.

Poster

712. Voluntary Movement and Motor Plasticity

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Topic: D.17. Voluntary Movements

Support: JSPS KAKENHI 15J03164

Title: Differential off-line effects of 10 Hz and 20 Hz transcranial alternating current stimulation on motor cortical excitability

Authors: *H. NAKAZONO, K. OGATA, S. TOBIMATSU;
Dept. of Clin. Neurophysiology, Neurolog. Institute,, Fac. of Med. Sciences, Kyushu Univ., Fukuoka-shi, Japan

Abstract: Background: Transcranial alternating current stimulation (tACS) has been considered to entrain the ongoing oscillatory activities in a frequency dependent manner. For the primary motor cortex (M1), 20 Hz tACS but not 10 Hz one increased the cortical excitability during tACS (online effect). However, it is unclear about the off-line effects of 10 and 20 Hz tACS on the M1 excitability. Thus, we investigated the plastic effects of these tACS over the M1. Methods: Twelve healthy subjects participated in this study. Alternating current of 1 mA was passed for 20 min between the left M1 and Pz electrodes. The frequency of tACS was set at 10 or 20 Hz, and the experiment of each frequency was conducted on separate days. M1 excitability was measured by the motor evoked potentials (MEPs) with single pulse transcranial magnetic stimulation (TMS). Two sessions of 12 MEPs were recorded before tACS as baseline. After tACS intervention, 12 MEPs were collected every 5 min up to 30 min. The mean amplitudes of MEPs after logarithmic transformation were compared before and after tACS. Results: MEPs were increased after 20 Hz tACS while they were decreased after 10 Hz one. These effects were short lasting, and returned to the baseline at 15-20 min after both interventions. Thus, the MEP trials of from 20 to 30 min after tACS were collapsed into 'over 20 min'. Statistical analysis showed that MEPs were significantly increased at 5-10 min after 20 Hz tACS while they were decreased at the same period after 10 Hz tACS. Discussion: We found not only the facilitation of the M1 excitability after 20 Hz tACS, but also inhibitory effect after 10 Hz tACS. These results suggested that both of 10 and 20 Hz tACS over M1 modulate the cortical excitability in the opposite direction, which last 5-10 min after intervention. The differential tACS effects of between 10 Hz and 20 Hz stimulation may results from the difference in the functional roles of alpha and beta oscillations in the human motor system.

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Poster

712. Voluntary Movement and Motor Plasticity

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Topic: D.17. Voluntary Movements

Support: NSERC

Title: Modulation of cTBS after-effects following aerobic exercise

Authors: *A. M. SINGH, W. R. STAINES;
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Abstract: Previous research indicates that acute aerobic exercise has a facilitatory effect on motor cortical excitability. Specifically, exercise may enhance the induction of early long-term potentiation (LTP) in the primary motor cortex, possibly via an increase in neurotransmitter release. Thus, exercise may be useful to prime cortical regions for plasticity when followed by more targeted interventions. However, the role of exercise when performed after the induction of plasticity has not been investigated. In addition, despite the potential for enhanced LTP-type processes, it is unclear whether the same effects are seen with techniques that induce long-term depression (LTD). Continuous theta-burst stimulation (cTBS) can be used to temporarily suppress excitability in the target region for up to 60 minutes. It is thought that this suppression is due to LTD-type processes occurring in cortical output neurons. Thus, in this study, we investigated the contribution of exercise to the duration and intensity of cTBS after-effects. In particular, we performed lower-limb aerobic activity following cTBS delivered to an upper-limb muscle representation. It was hypothesized that when exercise was performed immediately following cTBS, the duration and intensity of the effects of cTBS would be reduced. Healthy participants were recruited and underwent two experimental sessions: a) cTBS followed by rest, and b) cTBS followed by exercise. Following the identification of the motor hotspot for the right first dorsal interosseous (FDI) muscle, baseline measurements were collected, including resting motor-evoked potential (MEP) amplitudes, short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF). CTBS (600 pulses, 3-pulse bursts delivered at 5 Hz, 40 seconds) was then delivered over the FDI hotspot at 80% of the active motor threshold. Participants then underwent either 25 minutes of moderate-intensity stationary biking, or rested comfortably in a chair for 25 minutes. MEP amplitudes were measured at 3 timepoints following cTBS: 1 minute (just prior to exercise or rest), 30 minutes, and 60 minutes. SICI and ICF were also recorded at the 30-minute and 60-minute post-timepoints. Changes in MEP amplitude and the degree of SICI and ICF were quantified at each timepoint. Preliminary results indicate that exercise suppresses the effects of LTD-like plasticity when performed immediately following cTBS. This suggests that exercise may not necessarily enhance all types of plasticity induction, but rather favours the induction of excitability and may shift the balance of interneuronal network activity towards facilitation.

Disclosures: **A.M. Singh:** None. **W.R. Staines:** None.

Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 712.05/V46

Topic: D.17. Voluntary Movements

Title: The effect of aerobic exercise coupled with transcranial direct current stimulation on motor learning

Authors: *J. BAER, M. B. KOLAR, A. HARRISON, R. NEWMAN-NORLUND;
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Abstract: When treating a brain injury, such as during stroke recovery, traditional rehabilitation modalities typically involve periphery-driven increases in motor function that could be bolstered by central stimulation approaches. Transcranial direct current stimulation (tDCS) promotes motor learning by altering the excitability of cortical neurons. Anodal tDCS (AtDCS) increases the excitability of underlying neurons making them more likely to depolarize. Furthermore, a single bout of aerobic exercise can lead to increases in brain derived neurotrophic factor (BDNF). BDNF is a key player in CNS neuroplasticity with important roles in memory and learning that has been linked to benefits in motor learning. The purpose of the current study was to evaluate the effects of AtDCS and acute aerobic exercise on motor learning both in isolation, as well as in a combined approach where a single bout of aerobic exercise alters the level of BDNF which primes the neuronal environment creating the optimal conditions for subsequent AtDCS. We measured motor performance in the non-dominant upper extremity after healthy, young participants: (1) exercised for 20 minutes then received tDCS (combo), (2) exercised for 20 minutes then received sham stimulation (exercise), (3) rested for 20 minutes then received tDCS (stim), or (4) rested for 20 minutes and received sham stimulation (control). Our measures of motor performance included the Jebsen Taylor Hand Function test (JTHF), a series of 6 different locks (locks), and the Purdue Pegboard test (PPT). All three tests were administered at 3 different time points: baseline, immediately post treatment, and 30 minutes post treatment. We hypothesized that the greatest improvements in motor performance would be seen in individuals who performed 20 minutes of aerobic exercise followed by tDCS, and that participants in both the exercise and the stim groups would improve more than controls. A 3(time) x 4(group) mixed model ANOVA using JTHF times as the dependent variable was insignificant ($F_{3.96, 51.48} = .332$, $p > .05$). Similar ANOVAs using locks and PTT also failed to reach significance ($F_{4.28, 55.61} = .648$, $p > .05$; $F_{5.6, 72.78} = .938$, $p > .05$, respectively). Negative findings may be due to i) lack of sufficiently sensitive measures, or ii) individual variability regarding the influence of AtDCS. Carefully designed studies should include additional measures (serum BDNF, motor evoked potentials, BDNF genotyping) to clarify the exact effects of AtDCS, aerobic exercise, and possible interactions between the two. Additional research with measures more sensitive to motor learning may also help elucidate these effects further.

Disclosures: J. Baer: None. **M.B. Kolar:** None. **A. Harrison:** None. **R. Newman-Norlund:** None.

Poster

712. Voluntary Movement and Motor Plasticity

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Program#/Poster#: 712.06/V47

Topic: D.17. Voluntary Movements

Support: NYSDOH Contract C030173

Title: Targeted human cortical and spinal neuroplasticity by transpinal stimulation

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Abstract: In this study, we examined whether noninvasive cathodal or anodal transpinal constant current stimulation (c-tsCCS, a-tsCCS) can alter human cortical and spinal motor and reflex activity. Using noninvasive cortical, spinal, and peripheral nerve stimulation we examined the effects of 40-min of c-tsCCS and a-tsCCS on human motor evoked potentials (MEPs), sensory afferent-mediated MEP facilitation, transpinal evoked potentials (TEPs), flexor reflexes, cortical-mediated flexor reflex facilitation, and soleus H-reflex homosynaptic depression. We found that 40-min of c-tsCCS and a-tsCCS decrease the afferent-mediated facilitation of tibialis anterior MEPs. c-tsCCS increases and a-tsCCS decreases the cortical-mediated flexor reflex facilitation. Both c-tsCCS and a-tsCCS increased the cortical induced MEPs in the tibialis anterior muscle, but decreased the transpinal induced TEPs of hip flexors and increased the TEPs from ankle extesnors. c-tsCCS did not affect soleus H-reflex homosynaptic depression but decreased the flexor reflex size in the tibialis anterior muscle. Last, a-tsCCS decreased the flexor reflex size in the tibialis anterior muscle, and soleus H-reflex homosynaptic depression was not different from that observed after c-tsCCS. Our findings reveal that c-tsCCS and a-tsCCS have distinct effects on human cortical and spinal motoneuron activity.

Disclosures: **M. Knikou:** None. **L. Dixon:** None. **D. Santora:** None. **M.M. Ibrahim:** None.

Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 712.07/V48

Topic: D.17. Voluntary Movements

Title: Effects of prior hand use on practice-dependent plasticity and ballistic motor skill learning

Authors: *S. J. HUSSAIN, K. J. COLE;
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Abstract: The theory of homeostatic metaplasticity states that the history of postsynaptic activity shifts the threshold for induction of long-term potentiation and long-term depression, and these principles have been demonstrated in human motor cortex with respect to both exogenous (i.e., non-invasive brain stimulation) and endogenous (i.e., motor practice) activity. Some work has even shown that voluntary muscle contraction prior to brain stimulation can reverse the effects of the stimulation and increase the variability of responsiveness (Gentner et al. 2008; Goldsworthy et al. 2015). Further, the amount of hand use over 8 hours modulates the effectiveness of brain stimulation (Rosenkranz et al. 2014). Yet, the impact of prior hand use on motor learning and practice-dependent plasticity is unknown. Here, we aimed to replicate previous findings that decreasing hand use for 8 hours reduces corticospinal excitability and to also characterize the effects of reduced hand use on motor learning and practice-dependent plasticity. We recruited healthy adults for participation in a cross-over study. Testing sessions were divided into immobilization sessions and control sessions, and the order of sessions was counterbalanced. For immobilization sessions, subjects reported to the lab in the morning, completed a baseline assessment of ballistic thumb extension and were fit with a splint that restricted motion of the left thumb. Subjects were instructed to avoid left hand use while wearing the splint. 8 hours later, subjects returned to the lab, the splint was removed, and a recruitment curve for left opponens pollicis was collected. After baseline TMS, subjects repeatedly practiced the ballistic skill and another recruitment curve was collected after training. We also tested retention of the ballistic skill both 30 minutes and 24 hours after practice. During control sessions, all experimental procedures were identical except that subjects did not wear a splint at any time nor did they avoid left hand use. Preliminary results suggest that immobilization may impair acquisition of the skill but may also augment practice-dependent plasticity and retention. If these trends are maintained with further analysis, the results would suggest that short-term immobilization can selectively enhance retention of ballistic motor skills, potentially through an effect on practice-dependent plasticity.

Disclosures: **S.J. Hussain:** None. **K.J. Cole:** None.

Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 712.08/W1

Topic: D.17. Voluntary Movements

Support: DFG RE 2740/3-1

Title: Effects of anodal tDCS on motor skill learning in the acute phase of stroke: mechanistic and therapeutic considerations

Authors: P. HAUFLER, M. ROCHA CURADO, B. FRITSCH, *J. REIS;
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Abstract: Objective: Stroke is the leading cause of disability in adults. New therapeutic approaches are needed to improve impaired motor function. Anodal transcranial direct current stimulation (tDCS) can enhance motor learning in healthy subjects and chronic stroke patients. However , it is unknown, if tDCS is advantageous for motor skill learning as well as motor recovery in general in the acute phase after stroke. Methods: This ongoing study includes patients with acute, unilateral, first-ever ischemic stroke causing a moderate hemiparesis. The speed-accuracy-tradeoff function (SAF) is assembled on day 1, day 5 and day 35 after stroke. On day 1-5 patients practice the sequential visual isometric pinch force task (SVIPT1) with their paretic hand. Training is combined with either anodal tDCS or sham tDCS for 20 minutes over M1 of the affected hemisphere on day 2-4. The change from the initial SAF (day 1 to day 5 to day 35), motor skill learning over the first 5 days and retention on day 35 are compared between the sham-stimulated and anodal tDCS-stimulated patients. Moreover, the Grooved Pegboard Test (GPT) as well as a tracing task2 is performed on day 5 and day 35 to test for overall enhancement of motor function. Clinical scores are taken into account to assess neurological recovery in general. Results: At the time of this interim analysis patients showed similar initial impairment (UEFMS 58 ±2, NIHSS 5±1). On the training task, motor skill increased in both groups and was preserved at the day 35 follow up. Anodal tDCS-stimulated patients outperformed sham-stimulated patients, suggesting a beneficial effect of tDCS on motor skill learning in the acute phase. After training, patients showed a significant shift in the SAF, i.e. lower target error rates at predefined speeds. This shift was greater in the anodal tDCS group compared to sham. The anodal tDCS group also showed better performance on the GPT and tracing task. There was no difference between groups in terms of their clinical scores. Conclusions: Our preliminary data suggest that motor learning ability in acute stroke patients is preserved and that anodal tDCS combined with repeated motor training can facilitate motor skill learning in these patients. General enhancements in fine hand motor function indicated by the SAF shift, GPT and tracing task performance was also greater in the anodal tDCS group. These findings could be directly transferable to neurorehabilitative treatments of acute stroke patients as a safe strategy to facilitate recovery of lost motor function the first days after admission to a stroke unit. 1.Reis et al., PNAS 2009;106(5):1590-5. 2.Prichard et al., Brain Stimul. 2014 Jul-Aug;7(4):532-40.

Disclosures: **P. Haufler:** None. **M. Rocha Curado:** None. **B. Fritsch:** None. **J. Reis:** None.

Poster

712. Voluntary Movement and Motor Plasticity

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Program#/Poster#: 712.09/W2

Topic: D.17. Voluntary Movements

Support: CIHR MOP-130269

Title: Multimodal imaging to assess structural and functional changes associated with motor skill acquisition in healthy adults

Authors: ***B. LAKHANI**[1], M. R. BORICH[2], J. N. JACKSON[1], K. P. WADDEN[1], S. PETERS[1], A. VILLAMAYOR[1], A. MACKAY[1], I. VAVASOUR[1], A. RAUSCHER[1], L. A. BOYD[1]; [1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Emory Univ., Atlanta, GA

Abstract: Introduction: The brain undergoes numerous structural and functional changes when learning a motor skill. Multi-component T_2 relaxation imaging provides a validated marker of myelination (myelin water fraction or MWF). To date, no research has longitudinally quantified changes in myelin, cortical structure and functional connectivity in response to motor skill learning. Therefore, the objectives of the current study are to explore the relationship between changes in MWF and acquisition of an upper-limb motor task in healthy young adults and to identify concomitant changes in cortical volumetrics and functional connectivity. Methods: Seventeen healthy young adults (26 ± 4 years old; 10 female) underwent 3T magnetic resonance imaging before and after ten sessions of unimanual upper-limb training with their dominant right. MWF was extracted from the 32 echo T_2 relaxation MRI data using a non-negative least-squares with extended phase graph algorithm. Regions of interest for MWF analysis included the white matter underlying the intraparietal sulcus (IPS) and the parieto-occipital sulcus (POS). Cortical reconstructions and volumetric segmentation were performed using the FreeSurfer image analysis package. Resting-state fMRI (rsFMRI) analysis was conducted using independent component analysis with a spatial constraint seeded in the left IPS with the Group ICA function of the fMRI Toolbox. Skill acquisition was quantified by fitting an exponential curve to movement time. Results: The left IPS and left POS demonstrated significant MWF increases following training (8.30% and 6.28%, respectively). There was a significant correlation between the change in MWF in the left IPS and the change in cortical volume in the gray matter of the left IPS ($r=-0.697$, $p=0.003$). Consequently, there was a significant partial correlation between the percent difference in MWF in the left IPS and the rate of skill acquisition ($r=-0.607$, $p=0.016$),

when controlling for gray matter volume in the left IPS. In addition, rsFMRI contrast analysis identified one cluster located in the left middle frontal gyrus with a significant increase in activity following training (p=0.002). Discussion: This is the first demonstration of focal increases in MWF after skill training. Increases in MWF were observed only in ROIs contralateral to the trained limb, were correlated with indices of skill acquisition, and corresponded to regional decreases in cortical volume. Additionally, rsFMRI demonstrated changes in functional connectivity between the left IPS and the middle frontal gyrus, which is implicated in visuomotor attention.

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Poster

712. Voluntary Movement and Motor Plasticity

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: D.17. Voluntary Movements

Support: Canadian Institutes of Health Research

Title: Influence of inter-train interval on the efficacy of repetitive transcranial magnetic stimulation

Authors: ***R. CASH**[1], A. DAR[2], J. HUI[1], L. DE RUITER[2], C. BURKE[2], J. DOWNAR[2], R. CHEN[1];
[1]Toronto Western Res. Inst., Toronto, ON, Canada; [2]MRI-Guided rTMS Clinic, Dept. of Psychiatry, Univ. Hlth. Network, Toronto, ON, Canada

Abstract: INTRODUCTION: Previous studies indicate that incorporation of inter-train intervals (ITI) between trains of repetitive transcranial stimulation (rTMS) may be essential not only for participant safety, but also for inducing long term potentiation (LTP)- like increases in cortical excitability. We systematically investigated the influence of ITI duration on rTMS effects. METHODS: 10 participants were recruited thus far (aged 22 ± 1 years). rTMS (20Hz, 2 second train, 1200 pulses, 100% RMT) was delivered with 4 different ITIs (4, 8, 16, 32 seconds), in a randomised, cross-over design. The investigators performing the analysis were blinded to ITI used. Motor evoked potential (MEP) amplitude and short interval intracortical inhibition (SICI) were measured pre-and up to 75 minutes post-intervention. At baseline conditioning stimulus

intensity was adjusted such that SICI reduced MEP amplitude by ~50%. Electromyographic activity was recorded from four muscles including target muscle first dorsal interosseous (FDI). RESULTS: Preliminary results suggest that rTMS was most effective at ITI 4 sec, with the largest LTP-like effects, the lowest inter-individual variability, and the greatest muscle specificity. The average MEP increase over 75 minutes were: 4sec: 131 ± 11%, 8sec: 106 ± 9%, 16sec 110 ± 10% and 32sec 114 ± 13% compared to pre-rTMS baseline. SICI was maximally disinhibited at ITIs of 4 and 8sec ITI from a pre-rTMS level of ~50% inhibition to a post-rTMS level of ~30% inhibition. The effects on SICI was shorter lasting that the effects on corticospinal excitability. DISCUSSION: rTMS at ITI of 4 sec may be more effective at increasing cortical excitability than longer ITI. This preliminary result suggests that rTMS protocols used therapeutically may be shortened for greater efficacy and shorter session duration, leading to reduction in treatment cost and increase the number of patients that can be treated in a given time.

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Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 712.11/W4

Topic: D.17. Voluntary Movements

Title: Short-latency afferent inhibition differentially suppresses motor cortical networks depending upon visual attention load

Authors: *J. L. MIRDAMADI, L. Y. SUZUKI, T. R. ERICKSON, A. S. FEINGOLD, S. K. MEEHAN;
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Abstract: Primary motor cortex works in association with different brain areas to support different types of learning. We recently demonstrated that attention influences motor cortical plasticity through somatosensory gating. However, the motor cortical substrates of this interaction are not known. Here, we examined the magnitude of short-latency afferent inhibition (SAI) under differing attention load upon motor cortical I-wave networks. SAI was elicited with electrical median nerve stimulation at the right wrist that preceded a single monophasic TMS stimulus over the left motor cortical representation of the first dorsal interosseous (FDI). To delineate the magnitude of SAI upon different I-wave networks, unconditioned and conditioned

motor evoked potentials (MEPs) were evoked using either posterior-anterior (PA) or anterior-to-posterior (AP) current. Motor evoked potentials (MEPs) evoked by each current direction were recorded while participants engaged in a visual detection task of varying attention load. The visual attention task consisted of colored crosses presented in different orientations. Under low visual attention load, participants were required to count all red crosses regardless of orientation. Under high visual attention load, participants were required to count only upright yellow and upside down green crosses. We hypothesized 1) that SAI would be greater for PA compared to AP current direction during low visual attention load and 2) that SAI would be significantly reduced for the AP current direction but not the PA current direction under high visual attention load. Preliminary data (n=5) support our hypotheses. SAI was greater for MEPs elicited using the PA current direction compared to AP current. SAI was reduced under high attention load compared to low attention load for AP but not PA current. These results suggest that attention specifically alters cortical excitability of motor cortical circuits mediating late I-waves. Decreased SAI possibly reflects reduced sensory projections to M1 as a result of attention-related sensory gating. Late I-wave cortical networks within M1 have been linked to model-based learning involving the cerebellum. Attentional modulation of these same motor cortical networks may serve as a point of convergence for implicit and explicit model-based learning. Understanding the interactions between cerebellar-mediated implicit and attention-related strategic mechanisms has important implications for skilled learning.

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Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 712.12/W5

Topic: D.17. Voluntary Movements

Title: Activation training alters corticomotor excitability of the gluteus maximus

Authors: *Y.-L. KUO[1], C. M. POWERS[1], A. C. SOUTHAM[1], Y.-Y. LEE[2], B. E. FISHER[1]; [1]Biokinesiology and Physical Therapy, USC, Los Angeles, CA; [2]Healthy Aging Ctr., Chang Gung Univ., Taoyuan, Taiwan

Abstract: Objectives: To determine whether a short term (6 hours) activation training program targeting gluteus maximus (GM) results in neuroplastic changes in the primary motor cortex (M1). Study Design: Within subject - repeated measures Background: It has been proposed that

strengthening and skill training of GM may be beneficial in treating various knee injuries. Given the redundancy of the hip musculature and the small representational area of GM in M1, learning to activate this muscle prior to prescribing strength exercises and modifying movement strategy would appear to be important. Methods: Using Transcranial magnetic stimulation (TMS), motor evoked potentials (MEPs) were obtained in 12 healthy individuals at 5 different stimulation intensities while subjects performed a double-leg bridge. Subjects then completed a home exercise program for approximately 1 hour/day for 6 days that consisted of a single exercise designed to selectively target GM. Baseline and post-training input-output curves (IOCs) were generated by graphing average MEP amplitudes and cortical silent period (CSP) durations against corresponding stimulation intensities. Linear slopes of the IOCs were compared pre and post training using a paired t-test. Results: Following GM activation training, the linear slope of the MEP IOC increased from 14.89 to 21.51 ($p = .01$). For CSP duration, the linear slope of the IOC increased from 14.89 to 21.51 ($p = .04$). Conclusion: Short term GM activation training resulted in a significant increase in corticomotor excitability as well as changes in inhibitory processes of GM. We propose that the observed corticomotor plasticity will allow for better utilization of GM in the more advanced stages of a rehabilitation/training program.

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Poster

712. Voluntary Movement and Motor Plasticity

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Program#/Poster#: 712.13/W6

Topic: D.15. Basal Ganglia

Title: From motor pathway to song system - functional brain specialization in birds

Authors: *A. SIMON, S. LETZNER, O. GÜNTÜRKÜN;
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Abstract: Sven Ebbesson (1980) proposed in his parcellation theory that distinct neural systems evolve by differentiation and parcellation. These processes involve an increased specialization within neural circuits that is accompanied by a loss of neuronal connections within system components. In order to investigate if specialization of a primordial avian sensorimotor system is accompanied by a change of connectivity, we compared the global motor system of the non-oscine brain with the specialized and well-studied oscine song system. The oscine song system comprises of several telencephalic nuclei which are part of two major interconnected pathways:

the anterior forebrain pathway (AFP) and the posterior motor pathway (PMP). HVC, located adjacent to NCL, receives input from the ascending auditory system and links both of these pathways. Therefore, the HVC plays a key role within the oscine songs system. Within the HVC different projection neurons either target the robust nucleus within arcopallium (PMP), or Area X within medial striatum (AFP). Since the only three avian taxa that are capable of vocal learning (songsbirds, hummingbirds and parrots), show a remarkably similar song system, it seems not very likely that their vocal learning and production systems evolved completely independently. Indeed, a connectivity pattern resembling the one discovered in oscines also exists in pigeons and participates in the control of sequential movement patterns. Here we address the question if this motor control system could serve as a preliminary form of the oscine song system. According to the parcellation theory, we might expect that the PMP- or AFP-like neurons in pigeons are less specialized such that single neurons subserve both projection streams. In order to examine whether functional specialization is accompanied by such a change of neuronal connectivity, we injected different retrograde tracers into arcopallium and MSt in pigeons. Subsequently we scanned the pigeon NCL for biprojecting neurons which project to both MSt and arcopallium. These neurons might constitute a preliminary stage of specialized nerve cells in the HVC of songbirds. However, no biprojecting neurons, but a remarkable differentiated projection pattern to both areas were detected. Additionally, we discovered a topical projection from NCL to the intermediate arcopallium and found out that NCL projects only to the somatic MSt. These findings might indicate that these NCL neurons are already specialized. Altogether these results deliver new insights into the complexity of brain organization.

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Poster

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Topic: D.17. Voluntary Movements

Support: American Heart Association 14CRP18150008

IUPUI OVCR

Title: Influence of motor cortex stimulation plus motor training on neuroplasticity

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[1]Occup. Therapy, [2]Kinesiology, Indiana Univ., Indianapolis, IN

Abstract: Use-dependent neuroplasticity is an important factor for rehabilitation, yet the potential to influence neuroplasticity when combining with neuromodulatory techniques such as repetitive transcranial magnetic stimulation (rTMS) is not fully characterized. The objective of the study was to determine how engaging in motor practice during rTMS influences neuroplasticity. Ten young adults participated with a mean age of 24. Each participant completed three separate sessions in a random order: motor practice with sham-rTMS, motor practice with rTMS, and rTMS only. The motor practice protocol included 30 bouts of isometric contractions in a customized wrist device during rTMS or sham protocol. The rTMS/sham protocol was 30, 3 second trains at 10Hz with 70% resting motor threshold intensity. The intertrain interval was 30 seconds. Assessments included force steadiness and electromyography during a wrist extension task, the Box and Block Test (BBT), and transcranial magnetic stimulation (TMS) before and after the rTMS intervention. The force steadiness task was two trials of at least 10 seconds at 10% of the maximum voluntary contraction. The BBT is a measure of dexterity assessed as the number of small blocks moved in a minute. The TMS measures included 10 stimulations each at suprathreshold (116% of resting motor threshold), short-interval intracortical inhibition (SICI), and intracortical facilitation (ICF) with motor evoked potentials (MEP) recorded from the extensor carpi radialis (ECR) and extensor carpi ulnaris (ECU) muscles. Data were analyzed with a RMANOVA (time by condition) and change scores from baseline were used for correlation coefficients. The variability of force steadiness decreased after all three conditions ($p < 0.05$) and the amount of ECR activity significantly changed as well ($p < 0.05$). There was a significant time effect for the BBT ($p < 0.05$), with significant increases following the conditions with motor practice. The results from the MEP data were non-significant. There was a significant, negative correlation ($r = -0.61$, $p < 0.05$) between the variability of force with an increase in the BBT following the rTMS only condition, but not when motor practice was involved. These results suggest that a short intervention can influence motor control in young adults although these changes are modulated differently by motor practice plus sham-rTMS, motor practice plus rTMS, and rTMS alone. This demonstrates the importance of better understanding how possible rehabilitation interventions influence neuroplasticity and may need to be individually determined and prescribed in future contexts to maximize therapeutic potential.

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Poster

712. Voluntary Movement and Motor Plasticity

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Topic: D.17. Voluntary Movements

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NIH 5 T32 GM07108-40

Title: Paired stimulation induces spike-timing dependent plasticity of neural connections in primate sensorimotor cortex

Authors: *S. SEEMAN, B. J. MOGEN, E. E. FETZ, S. I. PERLMUTTER;
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Abstract: Classic studies have described spike-timing dependent plasticity (STDP) at synapses: the connection from neuron A to neuron B is strengthened (or weakened) when A fires before (or after) B within an optimal time window. Accordingly, spike-triggered stimulation between motor cortical sites in a behaving monkey resulted in changed motor output of the leading site. The ability to modify connections in cortex becomes useful in the context of injury when connectivity, and associated functionality, is compromised. To avoid the need for long-term, stable isolation of single units, one could control timed activation of two cortical sites with paired stimulation. We tested the hypothesis that STDP could be induced via prolonged paired stimulation as quantified by cortical evoked potentials (EPs). Two macaques were implanted with bipolar electrodes positioned in a grid over sensorimotor cortex bilaterally. At each site, one lead penetrated into cortex while the other rested on the surface. Bipolar stimulation at some sites evoked robust, short-latency EPs at other sites mediated by connections between the stimulation and recording sites. We performed conditioning sessions between interconnected sites (A and B) for 3 hours via a head-fixed device, the Neurochip, while the monkey engaged in free behavior in the cage. On different days we varied the delay between trains of 3 stimuli at A and B. Before and after conditioning, test pulses of various amplitudes were delivered to sites A, B, and a connected control site C that did not receive conditioning stimulation. The mean peak-to-trough amplitude of test EPs was quantified as a percent change in amplitude after conditioning compared to before. Previous STDP studies showed a 20 ms delay between activation of 2 sites is in the optimal range for inducing plasticity. Here, a 20 ms delay between stimulation at sites A and B produced a 78.1 ± 22.8% (mean ± SEM, n=3) increase in EP from A to B immediately after conditioning. This effect was site and direction specific: the EP from A to C (control) showed no effect (-4.2 ± 10.5%) and the EP from B to A (reverse direction) showed a slight depression (-14.3 ± 19.7%), as expected from STDP rules. Three hours after conditioning ended the EP from A to B remained larger by 54.2 ± 17.2% showing a lasting effect. Longer conditioning delays produced no effect (100 ms: -20.2% n=1, 200 ms: -10.4% n=1) indicating that paired stimulation follows STDP timing rules. Interestingly, paired stimulation did not induce plasticity between every pair of sites tested. Still, these results show promise for future

clinical applications to induce targeted plasticity in cortex, and elucidating connectivity of cortical networks

Disclosures: **S. Seeman:** None. **B.J. Mogen:** None. **E.E. Fetz:** None. **S.I. Perlmutter:** None.

Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 712.16/W9

Topic: D.17. Voluntary Movements

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Title: Corticospinal population activity during motor learning

Authors: ***A. J. PETERS**, J. LEE, S. X. CHEN, T. KOMIYAMA;
UC San Diego, La Jolla, CA

Abstract: The motor cortex controls movement through direct projections to the spinal cord. Despite this primary connection, the relationship between motor cortex activity and movement has been difficult to decipher. Moreover, extensive evidence has shown that the motor cortex is capable of significant plasticity which is engaged during and necessary for motor learning, indicating that this relationship may be flexible. We are investigating how these changes are manifested in population activity of the motor cortex of mice using two-photon calcium imaging. We employ a head-fixed lever press task which mice can learn over the course of two weeks. The same set of neurons are simultaneously imaged during behavior every day. Previously, we focused on the superficial input layers of the motor cortex and found that learning induces an initial period of highly variable activity, which is followed by stabilization into a novel but

reliable set of activity patterns corresponding to individual movements. We are currently contrasting this input layer activity with output activity of layer 5b neurons that project directly to the spinal cord. By comparing the activity of the primary input and output layers of the motor cortex, we are able to describe plasticity as it pertains to laminar specificity. We are currently testing the hypothesis that layer 2/3 exhibits substantial changes in the relationship between activity and movement while layer 5b corticospinal neurons are less flexible in this regard. Such a scenario would support the model that plasticity in this area is effectively an interlaminar phenomenon where networks of layer 2/3 cells serve to drive layer 5b corticospinal neurons that act as stable drivers of movement. If on the other hand corticospinal neurons exhibit activity as flexible as layer 2/3 neurons, then it provides evidence that given movements do not rely on consistent output from the motor cortex.

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Poster

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Support: MEXT/JSPS KAKENHI 25702033

MEXT/JSPS KAKENHI 26560282

MEXT/JSPS KAKENHI 23500617

MEXT/JSPS KAKENHI 26120002

Title: Plasticity of inhibitory effect on indirect cortico-motoneuonal pathways in humans

Authors: *T. NAKAJIMA[1], S. SUZUKI[1], G. FUTATSUBASHI[2], S. IRIE[1], T. KOMIYAMA[2], Y. OHKI[1];
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Abstract: We previously reported that repetitive combined stimulation (RCS) of pyramidal tract and peripheral nerve induces long-term potentiation (LTP) in indirect cortico-motoneuronal (C-M) excitations, which would be mediated via. cervical propriospinal neurons (PNs) in humans. In the current study, we examined plastic changes in inhibiotory systems in the spinal cord. Healthy volunteers, who all gave written informed consent, participated in the experiments.

Electromyograms were recorded from the right triceps brachii (TB) and extensor digitorum comunicis (EDC) muscles. RCS intervention (0.2 Hz) was performed for 10 min, during which transcranial magnetic stimulation (TMS) to the contralateral motor cortex was delivered in conjunction with the right superficial radial nerve stimulation (NERVE) at wrist, under weak contractions of the target muscles. Interstimulus interval (ISI) for the combined stimulation (CS) was set at 10 ms (TMS behind), which gave converging inputs in upper cervical segments. Under the ISI, stimulus strengths for CS were determined before RCS, at which TMS-induced motor evoked potentials (MEPs) were suppressed most by combined NERVE. MEP amplitudes in TB and EDC were significantly suppressed after RCS, which lasted for ~70 min. The suppression was observed in both MEPs evoked by TMS and transcranial electrical stimulation (TES). Furthermore, the NERVE-induced suppression on MEP was significantly enhanced after RCS than that before RCS. The suppression was not observed in the initial part of MEP. These results suggest that RCS can induce plastic changes in pyramidal inputs to spinal inhibitory systems, which receives peripheral nerve inputs and inhibits PN and/or motoneurons.

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Poster

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Support: NIH Grant R01 NS012542-40

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Title: Electrical cortical stimulation paired with volitional movement produces subsequent intra- and inter-hemispheric effects in the nonhuman primate

Authors: ***A. R. BOGAARD**[1,2], S. ZANOS[2], A. G. RICHARDSON[3], E. E. FETZ[2];
[1]Med. Scientist Training Program, [2]Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [3]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Pairing cortical stimulation (CS) with volitional movement can have effects that last beyond the end of stimulation. Documented effects depended on the timing of stimulation

relative to movement and included changes in the motor output of sites ipsilateral to stimulation. In those studies, stimulation was delivered only to sites contralateral to movement and analysis primarily described effects on movement contralateral to stimulation. Interhemispheric effects and the underlying neurophysiological mechanisms need further review. Two male macaques were trained to perform a reaction time (RT) task using bilateral wrist movements. Each wrist controlled a cursor on a screen. For each trial, one cursor was randomly cued to be moved by rapid wrist extension into a "target" box, while the other cursor remained still in a "resting" box. Each session consisted of 3 epochs of about 500 trials each: preconditioning, conditioning and postconditioning. During the conditioning epoch, brief trains of suprathreshold electrical CS were delivered to a motor cortex electrode at a given latency relative to expected movement onset. The behavioral effects of conditioning were assessed by comparing RTs of movements contralateral and ipsilateral to the stimulated hemisphere during the preconditioning and the postconditioning epochs. Field potentials were recorded bilaterally during the task. At baseline, average RTs were similar between the two monkeys (monkey I: 244 ms; monkey K: 236 ms). When CS was triggered by contralateral movement (cont-CS; e.g. left movement triggers right motor cortex stimulation), RTs of both contralateral and ipsilateral movements decreased (I, contra: -22.3 ms, ipsi: -58.8ms; K, contra: -28.5ms n.s., ipsi: -20.0ms). When CS was triggered by ipsilateral movement (ipsi-CS), RTs increased for ipsilateral but not contralateral movements (K, contra: 18.8ms n.s., ipsi: 6.15 ms). In agreement with previous studies, cont-CS decreased RTs of contralateral movement. Here, we observed an even more robust decrease in the RTs of ipsilateral movement. Our results also demonstrate an increase in RT of contralateral movements during ipsi-CS. Assuming that RTs are indicative of underlying cortical activation, these effects can be explained by excitation of cortical activity in the movement-generating hemisphere by cont-CS, and by an increase in interhemispheric inhibition, known to arise from the unrecruited hemisphere, by ipsi-CS. Analysis of neural signals collected during performance of the task will allow us to explore physiological correlates of these behavioral effects.

Disclosures: **A.R. Bogaard:** None. **S. Zanos:** None. **A.G. Richardson:** None. **E.E. Fetz:** None.

Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

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Topic: F.02. Animal Cognition and Behavior

Title: Tool-use learning in rats

Authors: *A. NAGANO[1], K. AOYAMA[2];
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Abstract: Tool-use behavior has been observed in non-human animal species such as keas and common marmosets. Tool use has been observed in the wild and in experimental settings. Some primates and a species of rodent (degus) have been used as animal models to investigate the neural basis of the tool-use behavior. However, a more efficient animal model is needed. In the present study, we tested whether rats could be trained to use tools to obtain food in an experimental setting. We used eight experimentally naïve male Brown-Norway rats, approximately three months old at the start of the experiment. During the training and testing phase, all rats were maintained at approximately 85% of their initial free feeding weight. Each daily experimental session consisted of 36 trials in each phase. The rats were trained to use a hook-shaped tool to retrieve a food (one eighth of a chocolate flavored loop cereal) beyond the rats' reach. In the training phase, rats had to choose between an appropriately arranged hook and an inappropriately arranged hook. If the rat pulled the appropriate hook directly, rats could obtain the food because the food was placed inside the hook. On the other hand, if the rat pulled the inappropriate hook, rats could not obtain the food because the food was placed outside the hook. This training continued until the rats reached a criterion of 30 or more successful trials in two consecutive sessions. All rats reached this criterion after 15 to 41 sessions. After the rats reached the criterion in the training phase, one test session was run for each rat. In the test phase, rats had to choose between a functional rake-shaped tool and an unfunctional rake-shaped tool. If the rat pulled the functional rake, the rat could obtain the food. In contrast, if the rat pulled the unfunctional rake, the food passed through the rake blade. We analyzed their choice between the functional rake and the unfunctional rake in the test phase with a chi-square test. This analysis revealed that all rats could choose the functional rake, which was above chance levels. The results indicate that rats could select functional tools based on features of the tools in an experimental setting. This is the first time tool use has been reported for the rat. Thus, we offer the rat as an animal model that can be used to investigate the neural mechanism of higher cognitive functions in animals and, potentially, humans.

Disclosures: A. Nagano: None. **K. Aoyama:** None.

Poster

712. Voluntary Movement and Motor Plasticity

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Topic: D.17. Voluntary Movements

Support: Acorda grant Dal-MECH-Car-1

Title: 4-Aminopyridine strongly increases motor cortex and spinal stimulation responses at a clinically relevant dose

Authors: *A. SINDHURAKAR[1], A. MISHRA[1], T. BETHEA[1], J. IACI[2], T. PARRY[2], J. B. CARMEL[1,3];
[1]Burke Med. Res. Inst., White Plains, NY; [2]Acorda Therapeut., Ardsley, NY; [3]Dept. of Neurol. and Pediatrics, Brain and Mind Res. Inst., New York, NY

Abstract: The potassium channel blocker 4-Aminopyridine (4-AP) improves walking speed in people with multiple sclerosis (MS) and in people with stroke. The mechanism of action of 4-AP in MS may be through reversal of conduction block, but how 4-AP acts to improve sensorimotor deficits after stroke is not known. The current experiments test which circuits mediate 4-AP effects at clinically relevant serum concentrations. We hypothesize that 4-AP will increase excitability of both descending motor connections and spinal cord circuits. We first determined the proper dose of 4-AP required to achieve plasma levels which were effective in human trials-60-100 ng/ml-in adult female Sprague Dawley rats. A dose-finding study indicated that a 4-AP dose of 0.8 mg/kg would produce these levels, and we used this dose to investigate which neural circuits are affected by 4-AP. All experiments were performed on anesthetized and uninjured rats. The effects of 4-AP on descending motor pathways were studied by stimulating the motor cortex and recording the motor evoked potential (MEP) generated from the biceps muscle. In the same animals, spinal excitability was assessed by stimulating the dorsal spinal cord and recording MEPs from the bicep. Rats received either 4-AP or saline by intraperitoneal infusion and the experimenter was blinded to the group. Cortical MEPs and spinal excitability were then evaluated and blood samples were obtained prior to dosing of 4-AP or saline and 0.25, 0.5, 1, 2, 3, 4 and 5 h thereafter. The average maximum plasma level of 4-AP was 103.9 ± 22.3 ng/ml, and these levels fell to 39.7 ± 11.4 at 3 hours after infusion. Both cortical and spinal stimulation responses were markedly enhanced with 4-AP. The MEP response to cortical stimulation increased by 76 ± 19% from baseline and returned to baseline levels at four hours after injection. Spinal excitability increased 27 ± 6% above baseline, and it also returned to pre-infusion levels by 4 hours. Compared with saline controls, 4-AP significantly ($p<0.05$) increased MEPs evoked from both cortical and spinal cord stimulation in naïve rats. In rats, a 4-AP dose that produces therapeutically relevant plasma concentrations was found to increase the excitability of motor circuits, including spinal excitability, a finding not reported previously. Ongoing experiments in rats with stroke will test whether 4-AP affects motor function after stroke by strengthening spared neural circuits.

Disclosures: **A. Sindhurakar:** 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.; Acorda Therapeutics. **A. Mishra:** None. **T. Bethea:** None. **J. Iaci:** A. Employment/Salary (full or part-

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Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 712.21/W14

Topic: F.02. Animal Cognition and Behavior

Support: NSERC-CRSNG

Title: Role of cAMP response element-binding protein and its target genes during motor skill learning

Authors: *B. OUIMET, G. BUREAU, M. CYR;
Dept. de biologie médicale, Univ. Du Québec À Trois-Rivières, Trois-Rivières, QC, Canada

Abstract: In humans and animals, learning a skilled motor task requires integration of many signals from different parts of the brain especially the basal ganglia, motor cortex, and cerebellum. It is well known that once learned, a motor skilled task is performed automatically and never totally forgotten. However, the underlying molecular mechanisms of this learning process are not well-understood. Our recent studies suggest a contribution of protein kinase A (PKA), extracellular signal-related kinases (ERK) and striatal-enriched protein tyrosine phosphatase (STEP) in motor learning. It is well established that cAMP respond element binding protein (CREB) is regulated by many kinases notably ERK and PKA, and is very important in cognitive learning and long-term memory. However, the genes activated by CREB, as well as their specific roles in motor skill learning, are not identified. In order to study a skilled motor task in mice, we used the accelerating rotarod, which is well known to reproduce the slower and faster learning phases. To investigate the role of CREB in relation to learning phases, we have evaluated the protein levels of CREB by western blot after each day of rotarod training in the striatum. We observed that CREB expression levels were increased after each day of training. Next, we have searched for specific genes activated by CREB binding. We performed chromatin immunoprecipitation (ChIP) of CREB and verified the levels of CREB binding on various genes related to synaptic plasticity (GluR1, cFos, Arc, BDNF, NR2B, PSD95) in the striatum of untrained and trained mice on the rotarod. We observed a tendency to increase in the binding of

CREB on genes NR2B, BDNF and PSD95. Other experiments will be needed to conclude our results. These results will be useful to better understand the mechanisms of encoding of the memory of a skilled motor task.

Disclosures: B. Ouimet: None. **G. Bureau:** None. **M. Cyr:** None.

Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 712.22/W15

Topic: F.02. Animal Cognition and Behavior

Title: Probing the role of motor cortex in motor skill learning

Authors: *S. B. WOLFF, B. P. OLVECZKY;
Harvard Univ., Cambridge, MA

Abstract: One of the most fundamental functions of the brain is to learn and generate movements. Its remarkable capacity to acquire and improve motor skills depends on the intricate interplay between a number of distinct brain areas. Their specific roles and interactions and the overall implementation of the learning process in neuronal circuitry are still not well understood. Motor cortex (MC) is a central player in this distributed motor network. To dissect its involvement in the acquisition and execution of complex, learned motor skills, we train rats in a lever-pressing task which requires learning spatiotemporally precise movement patterns. Using chronic lesions, we have previously shown that MC is dispensable for executing these learned motor sequences. In contrast, we now show that transient optogenetic perturbations of MC activity acutely disrupt the execution of these motor skills. Together, these findings suggest that MC has the capacity to modulate complex, learned motor behaviors, but may refrain from exercising this capacity once a motor skill is consolidated. This is in line with our previous finding that MC is, in fact, necessary for the acquisition of the skills we train. Based on these results, we hypothesize that input from MC to downstream motor areas serves as a teaching signal during skill acquisition, guiding the acquisition and refinement of subcortically generated motor sequences. To test this hypothesis and to identify a subcortical target for MC's 'tutoring', we are using molecular and viral tools to gain selective access to distinct projection pathways from MC to different downstream motor areas. Preliminary results suggest that striatum may be a target of MC's 'tutoring'. While chronic silencing of corticostriatal projections affects motor skill acquisition, transient silencing acutely degrades execution of the learned behavior. To further dissect the role of specific projections from MC to different downstream areas, we are

combining projection-specific targeting with high-throughput behavioral training, single unit recordings, optogenetics and pharmacogenetics.

Disclosures: S.B. Wolff: None. **B.P. Olveczky:** None.

Poster

712. Voluntary Movement and Motor Plasticity

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Topic: D.17. Voluntary Movements

Support: F31 HD078130-02

Title: Are changes in cerebellar excitability effector-specific?

Authors: *D. SPAMPINATO[1], P. A. CELNIK[2];
[1]Biomed. Engin., Johns Hopkins Unviersity Sch. of Med., Baltimore, MD; [2]Physical Med. and Rehabilitation; Neurol., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: One of the functions of the cerebellum in motor learning is to predict and account for systematic changes to the body or environment. This form of adaptive learning is mediated in part, by long-term depression of Purkinje cells in cerebellar cortex. We have previously shown in humans that changes in cerebellar-M1 connectivity, suggestive of cerebellar excitability, correlates with learning of a walking adaptation task. This was also found for a hand muscle (first dorsal interosseous or FDI) in association to visuomotor adaptation. However, the effector specificity of these findings is poorly understood. Using paired-pulse transcranial magnetic stimulation (TMS), we assessed inhibitory cerebellar-M1 connectivity (CBI) on healthy young adults, a measurement thought to represent the strength of cerebellar-to-cerebral pathways for a given muscle. In Experiment 1, we asked if learning a visuomotor rotation task with the right-hand elicit CBI changes to the right leg. We measured FDI and TA muscles before and after learning a visuomotor rotation with the hand. Additionally, we wanted to see if CBI changes were associated to the amount of learning that transferred to the right leg. In Experiment 2, we investigated whether simple movement execution (i.e. no learning) would produce effector specific CBI changes. To our knowledge, there are no studies that have investigated whether modulation of cerebellar-M1 connectivity occurs during the preparation of a voluntary movement. We assessed CBI for FDI muscle at five different time points during movement preparation of cued-simple reaction time (RT) task (Onset, 20, 40, 65, 90% of individual RT). The RT task was completed in two different sessions: (1) participants responded by moving the

FDI muscle and (2) participants moved the TA muscle while FDI muscle was kept relaxed. Our data shows that learning with the hand elicited changes in CBI for both FDI and TA muscles ($p<0.04$). This effect, associated to transfer of learned movements to the leg, cannot determine the somatotopic specificity of CBI. However, results from Experiment 2 revealed that CBI changes where only present prior to movement onset in the effector being moved (i.e. finger), but not the one at rest (leg). These results indicate that the cerebellar-M1 connectivity measure (CBI) is effector-specific. However, when the transfer of learning is present, CBI changes are also observed in the non-trained effector.

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Poster

712. Voluntary Movement and Motor Plasticity

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Topic: D.17. Voluntary Movements

Support: REM ANR-13-APPR-0008

Title: Updating eye-hand coordination when manipulating objects with complex dynamics

Authors: *F. DANION[1], C. LANDELLE[1], A. MONTAGNINI[1], L. PERRINET[1], L. MADELAIN[2];
[1]CNRS, INT, Marseille, France; [2]URECA, University of Lille, France

Abstract: The ability to track with the eye a moving target is substantially improved when the target is self-moved in comparison to an externally-moved one. In particular it has been shown that when the target is moved by the subject's hand, eye tracking is characterized by a higher gain in smooth pursuit, fewer corrective saccades, and a smaller temporal lag between target and eye motion. However all those observations were obtained when the mapping between hand and target motion was kept linear. Here we investigate a situation in which the mapping between hand movement and target motion is altered so as to mimic an elastic link between hand and target, thereby simulating that a weighted target is attached to the hand by means of a damped spring. In order to remain efficient, these changes in target dynamics require the update between hand actions and their visual consequences. Our objective was to determine whether eye-hand coordination could accommodate these rather complex (non-linear) changes in hand-target dynamics. To fully appreciate the effects of this (visual) perturbation, we have compared eye-tracking performance when moving a rigid and a non-rigid target, as well as when the subject

faces target trajectories that he/she had previously generated when self-moving the rigid or non-rigid target. As in previous studies, we showed that eye tracking is more accurate when the target is self-moved. However, although this advantage was immediate when dealing with the rigid target, this was not the case when exposed to the non-rigid target. Still within a few minutes of practice with the non-rigid target, tracking performance improved substantially in the self-moved condition, up to a level that was similar to when self-moving the rigid target. In contrast performance did not improve much when tracking an externally-moved target, even though tracking was consistently better with the non-rigid target. At this stage it seems that the narrowing of the frequency bandwidth toward a resonant frequency could contribute to the advantage of the non-rigid target. Overall, despite the possible contribution of this resonant frequency, our results show that subjects can learn, within a few minutes, a complex mapping between their hand motion and their visual consequences. Acknowledgments: This work was supported by a French National Grant: REM ANR-13-APPR-0008

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Poster

712. Voluntary Movement and Motor Plasticity

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Topic: D.04. Vision

Support: Swiss National Science Foundation

Title: Visuomotor coupling is necessary for the development of sensorimotor integration in mouse visual cortex

Authors: ***A. ATTINGER**, B. WANG, G. KELLER;
Friedrich Miescher Inst., Basel, Switzerland

Abstract: Neurons in primary visual cortex (V1) of the mouse not only respond to visual stimuli, but are also driven by motor-related signals. In mice navigating in a virtual reality environment layer 2/3 neurons strongly respond when the actual visual flow deviates from the visual flow that the animal expects as a consequence of its own locomotion. The detection of such mismatch events requires the integration of both visual and motor-related signals. It is still largely unknown how experience shapes this sensorimotor integration in V1. To assess this, we raised one group of mice in conditions of normal visuomotor coupling, in which forward locomotion

was coupled to backward visual flow in a virtual reality environment. A second group was raised in conditions of random visuomotor coupling, in which locomotion had no influence on visual flow. Both groups were raised in complete darkness when not in the virtual reality setup. After six two-hour training sessions on alternating days, we measured activity of defined populations of neurons in the superficial layers of V1 using *in vivo* two-photon imaging of a genetically encoded calcium indicator (GCaMP6f or GCaMP5) expressed in a Cre-dependent manner. Even though both groups of mice experienced identical visual input and exhibited indistinguishable locomotion behavior, we found profound differences in sensorimotor processing between the two groups. Selective responses to mismatch were only present in animals raised under coupled visuomotor conditions. These differences in processing were particularly prevalent in inhibitory neurons. Moreover, different classes of interneurons responded differently to mismatch events. Based on these data, we developed a circuit model of mismatch detection. Although absent initially in animals with random visuomotor coupling, selective responses to mismatch developed quickly with coupled visuomotor experience. These data indicate that normal development of V1 function critically depends on the experience of visual feedback coupled to movement.

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Poster

712. Voluntary Movement and Motor Plasticity

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Program#/Poster#: 712.26/W19

Topic: D.17. Voluntary Movements

Support: National Institute of Neurological Disorders and Stroke R01 NS085167

Title: Effects of vagus nerve stimulation paired with motor training on contralesional cortical plasticity after brain injury

Authors: *D. PRUITT[1], A. SCHMID[2], K. FLANAGAN[2], B. BAKER[2], C. ABE[2], R. MORRISON[2], J. TRIEU[2], S. SHAH[2], M. P. KILGARD[2], R. L. RENNAKER, II[2];
[1]The Univ. of Texas At Dallas, Richardson, TX; [2]The Univ. of Texas at Dallas, Richardson, TX

Abstract: Traumatic Brain Injury (TBI) is one of the largest health problems in the United States. The effects of TBI can be debilitating and recovery of function is often incomplete. We previously demonstrated that vagus nerve stimulation (VNS) paired with forelimb use enhances motor recovery after TBI. Here we have investigated how varying the parameters of VNS affects

both functional motor recovery and contralesional cortical plasticity after TBI. Rats were trained to pull on a handle to receive a food reward, and then received a controlled-cortical impact (CCI) in motor cortex. After CCI, animals were assigned to experimental groups receiving VNS at differing levels of current (0.8 mA or 0.4 mA) either paired with motor training or delivered separate from motor training. Following six weeks of post-lesion training, we investigated contralesional cortical plasticity using intracortical microstimulation in the motor cortex. Our findings indicate that motor training alone enhances contralesional cortical plasticity, but no effect of VNS is observed in the contralesional hemisphere.

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Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

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Program#/Poster#: 712.27/W20

Topic: D.16. Posture and Gait

Support: DFG (Exc 257)

Title: Motor and limb performance assessment with OptiMan an operator-independent fully-automated multi-sensor system for forelimb strength, gait performance, motor coordination and motor learning assessment

Authors: ***Y. WINTER**[1], H. MUNAWAR[2], W. CLEMENT[3], C. JUNG[4], C. REIMERTZ[3], M. RIVALAN[2];
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Abstract: Asssessing motor performance of animals by means of behavioral test is often influenced by operator-animal interaction and represents a stressful situation for the animal that may largely influence test results. In order to analyze motor phenotypes that are specific for a given disease/deficit we developed and validated a battery of automated behavioral tests that

allows the continuous assessment of motor function of rats in a fully operator-independent setup. Four to six RFID-tagged rats are group housed in a home cage environment. Their individual motor activity including locomotion distances and speeds is continuously monitored with a RFID-sensor array placed underneath the home cage. Rats can voluntarily leave their home cage by passing through an RFID-based electronic gating system and individually enter a course of four sequential units. First, a weighing cage to record body mass, secondly followed by a horizontal ladder task (1 meter) with electronically monitored rungs to evaluate limb stepping, placing, and coordination. Thirdly, an operant cage comprising a bilateral isometric pull task that evaluates forelimb function and strength while the animal learns to pull a retracted lever to obtain pellet reinforcement. The fourth and last unit before the animal enters back into its home cage is a walkway consisting of a high-density piezo force transducer array assessing gait kinematics and individual limb weight bearing. Motorized gates and unidirectional flaps guide the animal through the multiple-chamber system in a one-way direction. We used this system with rats from different experimental backgrounds and demonstrate that different types of motor impairment yield significant differences in multiple parameters that can be measured in the OptiMan system. This operator-independent, home cage based test-battery yields objective, stress free high-quality motor function data that is comparable to or better than conventional behavioral testing and unprecedented in terms of quantity of data gained per time in a quasi-continuous fashion.

Disclosures: **Y. Winter:** None. **H. Munawar:** None. **W. Clement:** None. **C. Jung:** None. **C. Reimertz:** None. **M. Rivalan:** None.

Poster

712. Voluntary Movement and Motor Plasticity

Location: Hall A

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Program#/Poster#: 712.28/W21

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 5R01MH081153

Title: Betasort: A computation model of serial learning

Authors: ***G. G. JENSEN**[1], F. MUÑOZ[2], Y. ALKAN[2], V. P. FERRERA[2], H. S. TERRACE[1]; [1]Psychology, [2]Neurosci., Columbia Univ., New York, NY

Abstract: Do animals learn by purely associative mechanisms, or do they rely on representational structures? The success of “model-free” reinforcement learning (RL) in

explaining many complex tasks supports the former view. However, there are some tasks that seriously challenge RL models. A particularly salient example is the transitive inference (TI) task, which requires that subjects infer the ordering of a set of stimuli from incomplete information. TI is notable not only for the difficulty it poses for computational learning methods, but also for its ubiquity in comparative cognition: Every vertebrate species tested to date has demonstrated some aptitude for TI. Another cognitive task that is difficult to account for in associative terms is the simultaneous chain procedure (SimChain). We propose a novel computational model for serial learning, called betasort, which uses Bayesian updating to maintain a spatial representation of the stimulus order. Betasort readily and rapidly performs both TI and SimChain. We used betasort and Q-learning (a powerful RL algorithm) to fit behavioral data from 3 rhesus monkeys and 19 humans performing a 7-item TI task in which they were trained on adjacent pairs and tested on all pairs. Betasort demonstrated transfer of learning from adjacent to all pairs whereas Q-learning failed to learn during adjacent pair training. We also used betasort to fit data from 7 monkeys and 21 humans performing a 5-item simultaneous chain task; in this case, betasort yielded learning curves similar to those observed in subjects. In both of these tasks, betasort not only identifies correct responses, but also displays symbolic distance effects in its response errors, consistent with the hypothesis that serial learning makes use of a spatial continuum. Despite its representation of this continuum, betasort is efficient, able to simulate behavior at low computational cost. This sets it apart from Markov decision process (MDP) approaches, which often invoke intractable quantities that must be approximated numerically. Additionally, although reward prediction error (RPE) has long been assumed to play a pivotal role in neural networks, systems whose learning is determined by RPE signals, such as Q-learning, cannot approximate behavior in rigorous serial learning tasks. Betasort's success (when compared to RPE models) and its computational efficiency (when compared to MDP models) suggests that the debates over the "model-based vs. model free" dichotomy, borrowed from the machine learning literature, may have become a distraction from a central aim of modeling learning: To predict the behaviors that organisms display under controlled conditions.

Disclosures: G.G. Jensen: None. **F. Muñoz:** None. **Y. Alkan:** None. **V.P. Ferrera:** None. **H.S. Terrace:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: MSMT project LH14053 KONTAKT II.

Title: A novel test “Arenomat” for studying spatio-temporal integration

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Abstract: We would like to present an unique novel task developed in our laboratory for studying spatio-temporal integration in rats. As well as we are able to orientate in the space we also continually perceive time. For us it’s important to process the changes in this moving and diverse world and therefore we have to use these informations together. However yet this spatio-temporal integration hasn’t been studied extensively. For studying spatio-temporal integration, particularly integration of space and interval timing, we have developed a specialized tracking program called Arenomat. For our tasks we have applied classical behavioral apparatus - “Carousel” maze which is used to test animal’s spatial navigation and cognitive coordination. The apparatus consists of rotating arena with camera tracking animal’s movement. In original active allothetic place avoidance (AAPA) task is the animal trained to avoid aversive sector (usually 15 degrees wide) where a mild aversive stimuli is applied. Using Arenomat allows us to create novel and unique tasks which are analogs of AAPA task and allows us to test integration of spatio-temporal information. This include rotation of arena (different speeds and directions), precise timing of all processes, offering positive rewards (e.g. food pellet), negative stimuli (e.g. white noise, electric shock) and generating any kind of light or sonic stimuli. This novel test offers an insight into the cognition and behavior of rats during spatio-temporal integration and can be possibly a tool for studying neuronal mechanisms underlying this cognitive capacity. This work was supported by MSMT project LH14053 KONTAKT II.

Disclosures: K. Maleninska: None. **A. Stuchlik:** None.

Poster

713. Brain Machine Interfaces: Invasive Applications

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Topic: D.18. Brain-Machine Interface

Support: NIH Grant R01 NS048845

Title: Cortical control of co-contraction and its use for the regulation of stiffness in brain-machine interfaces

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Abstract: When interacting with different mechanical environments, humans regulate arm impedance in order to optimize stability and metabolic energy consumption. In 1982, Donald Humphrey described two distinct sets of neurons in the primary motor cortex (M1) of monkeys, one that was activated during reciprocal muscle activation, the other to control co-contraction. Brain-machine interfaces (BMI) have been successfully used to "decode" intended movement kinematics, and control robotic arms, but the users typically do not have control over impedance. We developed a BMI that controlled the activity of a virtual arm with a pair of simulated muscles spanning a single joint. The monkey learned to regulate the stiffness by co-contracting, but it did so using the same neurons involved in reciprocal contractions. We recorded neural activity from M1 and EMG activity from triceps and brachioradialis while a monkey generated isometric contractions of one or both muscles. We built a Wiener filter M1 to EMG decoder during a series of reciprocal muscle activations. During experiments, we fed the decoder outputs to the virtual dynamic arm. The torque applied to the joint was derived from the difference in activation between the two decoded muscles, with angular stiffness proportional to the co-contraction of the muscles. External forces could be applied to the endpoint of the virtual arm to displace it. When comparing neural activity between reciprocal and simultaneous muscle contractions, we were unable to find "co-contraction" neurons. Instead, we found a single population of neurons that was modulated during both reciprocal and simultaneous activation of the recorded muscles. The firing rate of single neurons did not modulate exclusively with the activation of any individual muscle. The monkey used the BMI to perform a task similar to Humphrey's, controlling the virtual arm as sinusoidal forces of different frequencies were applied to the endpoint. At a low frequency (0.25 Hz), the monkey was able to counter the perturbation by activating the simulated muscles reciprocally, with minimal co-contraction. At a high frequency (2 Hz), the monkey resorted to co-contracting, to increase the stiffness of the virtual arm. Implementing this approach to the control of a dynamic limb would allow BMI users to regulate the impedance of a robotic arm, making interactions with their environments safer and more natural.

Disclosures: R. Ruiz-Torres: None. **L.E. Miller:** None.

Poster

713. Brain Machine Interfaces: Invasive Applications

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Topic: D.18. Brain-Machine Interface

Support: NIH NS065186

NS 079200

NSF EEC-1028725

Title: Concurrent independent brain-computer interface and movement control from the same cortical site

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Abstract: Brain-Computer Interface (BCI) control can be disturbed by simultaneous movements that may be accompanied by neural activity in the same brain areas that are used for BCI control. Here we investigate whether it is possible to dissociate these competing activities and allow concurrent movements and BCI control. Furthermore we examine whether this concurrent control is accompanied by motor cortical reorganisation allowing for distinct cortical sites responding primarily to the BCI and less to movement behaviour. We present results from human ECoG studies where subjects perform concurrent BCI and movement control tasks, but where the same motor cortical region was used for both kinds of control. The channel over motor cortex most highly activated by finger movement in the contralateral hand was established in an initial screening. In the task this same finger movement controlled the horizontal movement of a computer cursor, whilst the mean power in the high gamma (70-90Hz) band from this same channel controlled the vertical movement of the cursor. Subjects had to move the cursor in a centre out paradigm to 8 targets spaced evenly on a circle. To reach the targets subjects had to learn to modulate the power in the band independently to the neural modulation that generated the finger movement. Subjects were able to gain this concurrent control indicating a dissociation of activity from the two behaviours at the control site. To examine if the BCI task caused any changes to the underlying activity at the control site subjects performed a force matching task by repeatedly gripping a force bulb in the fingers before any exposure to/and immediately after the BCI sessions. We compared the difference in activity for the force matching task pre and post BCI sessions and found differences in activity during the task after the BCI session compared to before the BCI session. This difference was confined to the control channel and occasionally

channels immediately around it also modulated during the task. The difference was most pronounced in the frequency band used for BCI control (70-90 Hz) while absent in many other bands also modulated during the task (e.g. beta 12-30Hz). Our preliminary findings demonstrate the potential for concurrent BCI and motor tasks and signs of motor cortex reorganization following this concurrent control, consistent with a re-assignment of activity from hand to BCI control, specific to the features used for BCI control. Future experiments aim to further refine this result.

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Poster

713. Brain Machine Interfaces: Invasive Applications

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Program#/Poster#: 713.03/W25

Topic: D.18. Brain-Machine Interface

Title: Neural responses to Activity-Dependent Stimulation (ADS) within different cortical areas

Authors: *A. AVERNA[1], D. GUGGENMOS[2], C. DUNHAM[3], S. BARBAY[3], G. VAN ACKER[4], M. CHIAPPALONE[1], R. NUDO[5];
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Abstract: Behavior is driven by the complex interactions of neural activity within and between brain regions. Neurological disorders, such as stroke, disrupt this neural communication and presumably, nervous system reorganization is required in order to recover function. Facilitating functional recovery following stroke and other cortical lesions remains an ongoing clinical challenge. The post-injury synaptic plasticity related to the recovery process occurs spontaneously after a brain lesion and can be aided by electrical stimulation to modulate firing thresholds and promote neural activity. One technique, activity-dependent stimulation (ADS), utilizes the neural activity recorded from one site as a trigger for electrical stimulation at another site and has shown success in promoting behavioral recovery by re-establishing an artificial connection between somatosensory and pre-motor cortex following primary motor cortical injury. This technique is based upon Hebbian neural conditioning in which neurons that fire synchronously have an increased probability of enhancing connectivity through long term

potentiation (LTP) or long term depression (LTD) of existing synapses, and potentially, to create new connections through axodendritic sprouting. It remains unclear how pairing neuronal populations using ADS will impact their firing properties. The aim of the present work is to investigate whether or not ADS can be used to potentiate functional connectivity between distant cortical locations in healthy brain within a single four hour recording session in ketamine-sedated rats. Spontaneous and evoked activity within the rat pre-motor cortex was recorded using a sixteen-contact electrode and analyzed following either ADS or Gaussian (randomized) distributed stimulation approximating the frequency observed in ADS condition (trigger channel, ~7Hz). Based on known differences in anatomical connectivity, two cortical areas within S1 (forelimb responsive area or barrel field; FL or BF) were chosen for comparison of both spontaneous and stimulus-evoked firing. The results indicate that ADS was more effective than the randomized stimulation in modulating both spontaneous and evoked activity, further strengthening the idea that ADS could promote and potentiate cortico-cortical connectivity. Moreover this effect appears to be greater when the ADS stimulation occurs in BF rather than FL. These results are critical for understanding the electrophysiological effects of ADS, as well as providing information to optimize ADS for treatment following brain injury.

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Poster

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Title: Brain machine interface control through neurofeedback guided beta rhythm modulation

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Abstract: Brain-machine interfaces (BMIs) are an emerging medical treatment for many forms of motor impairments, including paralyzed and “locked-in” patients. Most BMI studies concentrate on decoding intended movement parameters within experimenter defined control

periods, when it is assumed the subject wants to move. However, the ability of the user to volitionally start and stop BMI control periods is also a critical requirement for BMI usability. Volitional control of the beta sensorimotor rhythm (SMR) could be used as a "brain switch" by the subject to indicate when the BMI decoder should start or stop decoding. We present a proof of concept that mice can use graded neurofeedback to increase beta SMR power in motor cortex. Freely moving mice in a neurofeedback task received water reward by controlling an auditory pitch cursor. The cursor was controlled through a modified beta LFP power measure from intracortical electrodes in vibrissal motor cortex. Beta power (13-30 Hz) was normalized by broadband activity to reduce motion artifacts. Bilateral EMG and slow speed video were used to confirm motor activity. Preliminary results show that: (1) behavioral performance improves over sessions; (2) post-hoc spectral analysis of the local field potential confirms that online beta power estimation is consistent with a true beta SMR; (3) beta activity is correlated to EMG activity, confirming the beta SMR's association with motor function; (4) mice are able to volitionally modulate beta power, shown by contrasting pre- and post-brain control sessions; and, (5) high beta activity predicts elevated EMG activity. This work illustrates the feasibility of using neurofeedback to create a beta SMR "brain switch" for motor BMIs.

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Poster

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DARPA HDC HR011-14-C-0102

Title: Semi-chronic chamber system for multi-scale electrophysiology in non-human primates

Authors: *A. L. ORSBORN[1], C. WANG[2], K. CHIANG[2], M. M. MAHARBIZ[3], J. VIVENTI[2], B. PESARAN[1];
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Abstract: Electrophysiology can monitor neural activity across many spatial scales_from highly localized firing of individual neurons to large-scale field potentials like electrocorticography (ECoG). Each signal may be useful for capturing different aspects of neural processing, but how measures at different spatial scales relate to one another is poorly understood. For instance, the sources of neural activity contributing to ECoG signals are unclear. Simultaneous multi-scale recordings will be critical for understanding relationships between individual neurons and larger-scale brain networks, and their links to behavior. Here, we present a technique for multi-scale electrophysiology and preliminary data exploring the relationships between spiking activity, local field potentials (LFPs), and ECoG in the cortex. We developed a semi-chronic chamber system to simultaneously record from subdural μECoG and a penetrating microdrive (Gray Matter Research) in non-human primates. The microdrive has independently-movable electrodes, providing 3D sampling of cortical volumes. The design is modular, scalable, and compatible with electrical and optical stimulation techniques. Our approach uses silicone artificial duras (ADs) for chronic subdural μECoG recordings and to align the ECoG and microdrive components within 250 μm tolerance. μECoG arrays are encapsulated within the AD. Impedance measurements show that this embedding procedure has minimal impact on recordings. The ADs (without an array) also provide optical access, transmitting 75-80% of 473nm light. We used this system to study the relationship between spike, LFP, and ECoG signals across cortical depths. We recorded from the post-central gyrus in an acute, anesthetized preparation from one non-human primate (macaca fascicularis). μECoG was recorded with a custom polyimide array with 5 μm thick Copper, 3 μm Nickel, and 150 μm Gold traces and contacts. The array had 124 contacts (200 μm in diameter; 0.75 - 1.5 mm spacing) and 32 holes (500 μm diameter) for the microdrive electrodes (glass-coated Tungsten micro-electrodes; 1.5mm spacing) to pass through. Spiking, LFP, μECoG signals were recorded as drive electrodes were lowered through the cortical tissue. Interestingly, coherence analyses of LFP and μECoG across depths suggest that μECoG may capture neural activity across multiple cortical depths in a frequency-dependent manner, rather than reflecting a single superficial source. Future work will focus on integrating optogenetic stimulation and increasing the scale of recordings to achieve large-scale multimodal, multi-scale recording and manipulation of neural circuits to study behavior.

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Poster

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Topic: D.18. Brain-Machine Interface

Support: DARPA SUBNETS

Title: Stimulation in primate caudate nucleus modulates action selection in probabilistic reward task

Authors: *S. R. SUMMERSON[1,2], P. KHANNA[3], E. L. RICH[2,4], J. D. WALLIS[2,4], J. M. CARMENA[1,2,3];
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Abstract: The mesolimbic and basal ganglia are crucial in arbitrating how sensory information, experience and motivation guide behavior. As such, the basal ganglia have been shown to play a role in decision-making and voluntary action. When this neural circuitry is operating in an aberrant manner, it can give rise to certain neuropsychiatric conditions, such as addiction and post-traumatic stress disorder. In this context, the reinforcement learning framework is useful for studying how decision policies are developed and, more importantly, how they can be biased. Deep brain stimulation is a potential therapy for altering decision policies and developing restorative and permanent treatments for those suffering from neuropsychiatric conditions. Here we ask how the value of alternative actions may be biased when electrical stimulation is administered in the caudate nucleus (Cd), part of the dorsal striatum. The striatum, is known to encode the value of alternative actions. In particular, Cd neurons encode action and decision values which are updated over time as more experience with the set of possible actions is gained. Using the nonhuman primate (NHP) animal model, we study how stimulation of the caudate in the time period preceding action selection influences subsequent decisions. Subjects are trained in a probabilistic reward task with both instructed and free-choice trials, where a trial consists of holding a computer cursor at a center target for a fixed length of time and then moving the cursor to a peripheral colored target. Each colored target has an associated probability of reward which is learned by the subject through exploration. During the center hold time, we expect that Cd encodes values of upcoming actions. Therefore, we delivered high-frequency stimulation during the hold time,on instructed trials to the lower value target. This stimulation is shown to significantly increase the likelihood of selecting the lower value target during subsequent free-choice trials relative to sham stimulation. Moreover, the effect is even greater if the action selected results in reward. We modeled the effects of temporally specific electrical stimulation in Cd with temporal difference learning models, and found that stimulation introduces a bias term in the model which results in a shifted action selection policy. This work supports further studies of the neural substrates of volition. Developing this vital understanding of decision making processing and how decision making can be mediated through stimulation will help inform novel closed-loop deep brain stimulation therapeutic approaches for treating people with neuropsychiatric conditions.

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Poster

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DARPA SUBNETS Program

Title: Phase-dependent coding of decision information in posterior parietal cortex

Authors: ***D. J. HAWELLEK**[1], Y. T. WONG[1,3], N. D. DAW[1,2], B. PESARAN[1];
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Abstract: The posterior parietal cortex (PPC) plays a central role in integrating visual sensory information and upcoming actions. Firing rates of neurons in the PPC encode movement choices that animals make along with decision related variables such as expected value or confidence. Another hallmark of the PPC are rhythmic excitability fluctuations - the beta rhythm (~15Hz). Across the PPC and other sensory-motor structures neuronal spiking is robustly coupled to frequency specific phases of the local field potential (LFP). These two lines of observations have so far mostly been studied in separation, leaving a core question about PPC function unanswered: How do the encoding properties of neurons as seen in their spiking relate to the large-scale oscillatory network behavior? To address this we recorded single units and fields in the lateral and medial banks of the posterior intra-parietal sulcus while rhesus macaques engaged in a decision making task. More than 70 percent of all recorded neurons (74% monkey C, 71% monkey R, permutation test) contained significant mutual information (MI) about an upcoming movement choice in their firing rates. Likewise about 70 percent of all recorded neurons (69% monkey C, 68% monkey R, permutation test) showed significant spike-field coherence. Critically however we found that the MI neurons carried about an upcoming choice was modulated by the phase of the local field potential. We compared the MI about choice that

neurons contained in the firing rates at different phases of the beta oscillation to the MI obtained at random phases with identical sampling statistics. We found that MI is significantly non-uniformly distributed across LFP phases ($p<0.05$, permutation test). In addition, individual phase bins at around the trough of the beta oscillation showed significantly more MI (27.6% monkey C, 40.9% monkey R) than spiking at random phases ($p<0.05$, permutation tests FWER corrected). Interestingly, while spike count was symmetrically distributed around the spike-preferred phase, MI about choice was not and reached its peak slightly later (60°, monkey R). Our findings show that firing rate encoding properties of neurons in the PPC and large-scale circuit dynamics of sensory-motor structures are fundamentally linked. The encoding of sensory-motor decisions is temporally patterned by the beta rhythm. These findings have important implications for understanding the propagation of information in large-scale circuits. Furthermore, the design of brain-machine interfaces that target neural decision-making systems may benefit from incorporating neural time by closed-loop stimulation based on the LFP.

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Poster

713. Brain Machine Interfaces: Invasive Applications

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Title: Decoding arm movements using epidural ECoG in non-human primate

Authors: ***H. CHOI**[1], J. LEE[1], K. MIN[1], K. AHN[2], K. LEE[2], D. JANG[1];
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Abstract: The spatial resolution of electrocorticogram (ECoG) is known to be much higher than electroencephalogram. Recently, several studies have reported usability of epidural electrocorticogram (eECoG) for brain computer interface (BCI). eECoG is less invasive, and less inflammatory than traditional ECoG (subdural ECoG). However, the feasibility and performance of eECoG on BCI were not fully evaluated yet. In this study, we verified the usability of implanted eECoG in non-human primate by decoding the three dimensional arm trajectories using eECoG signals. Two micro electrode patches (32 channels) were inserted over duramater

on rhesus monkey's brain covering premotor cortex, primary motor cortex, and primary somatosensory cortex. Three motion sensors (IMU: Inertial Measurement Unit) were attached shoulder, upper arm, and lower arm. The monkey performed four directional arm movement tasks responding to target's location change (45, 135, 225, 315 degrees). We contemporary recording the arm movement signals and brain signals for 1hour, twice a week. The signal was analyzed off-line using MATLAB. To computing the correlation coefficient between the arm movement and brain signal, we used regression algorithm, time frequency power of the brain signals, and Cartesian (xyz) position of the movement signals. As results, some frequency bands of the brain signals were significantly correlated with the arm position and arm movement preparing. So we verified the eECoG's feasibility and showed significant and stable decoding performance over several months. This could support the efficacy of BCI using eECoG and the various neuro-prosthetics fields.

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Poster

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Title: Changes in functional connectivity due to brain-computer interface learning

Authors: K. CASIMO[1], *K. E. WEAVER[2], J. D. WANDER[3], A. KO[4], J. G. OJEMANN[4], F. DARVAS[4];
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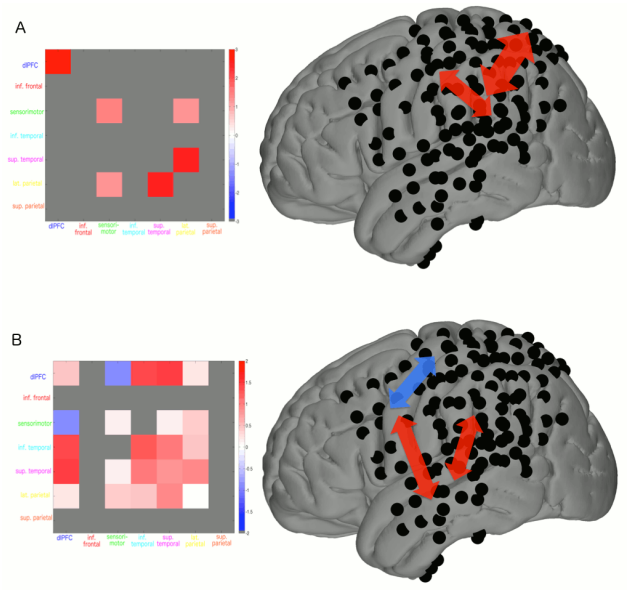
Abstract: Prior work on brain-computer interface (BCI) learning mechanisms has focused on changes in connectivity of the BCI-driving site during the task itself. Connectivity between a motor cortex BCI-driving site and distant sites has been observed. However, patterns between those distant sites have not been characterized, and the persistence of these changes after the BCI task has not been examined. We used phase coherence, also called phase locking value (PLV), to evaluate changes in resting state connectivity after BCI learning between functional anatomical

regions including and beyond the BCI site. Participants were trained to use a challenging two-dimensional BCI with electrocorticographic (ECoG) electrodes implanted directly on the surface of the cortex. Electrode position was controlled with modulation of high gamma (HG, 70-200Hz) power in hand motor cortex controlled vertical position of the electrode. After BCI learning, we found significant increase in beta (13-30Hz) PLV between sensorimotor (SM) cortex (location of the BCI control site) and lateral parietal lobe, within the parietal lobe, and between other known skill-learning network sites (Figure, A). There were significant increases in HG PLV within SM and within parietal cortex, but not between SM and parietal cortices. Notably, this is a broad HG range (70-200Hz), such that these effects would not be seen in traditional coherence measures. We also evaluated slow (<1 Hz) amplitude modulation of HG, a marker of stable cortical networks (Ko et al., 2013). We found significant increases in frontal-temporal and temporal-parietal PLV and significant decrease in SM-dlPFC PLV (Figure, B). Persistence of changes in connectivity after the task implies that the observed changes are due to robust skill learning and consolidation. Control site connectivity to learning-related regions and increases in connectivity between regions other than the BCI site further support the view that BCI skill acquisition depends on a widespread network of cortical regions that interact directly besides their interactions with the BCI control site.



Disclosures: **K. Casimo:** None. **K.E. Weaver:** None. **J.D. Wander:** None. **A. Ko:** None. **J.G. Ojemann:** None. **F. Darvas:** None.

Poster

713. Brain Machine Interfaces: Invasive Applications

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 713.10/W32

Topic: D.18. Brain-Machine Interface

Title: Towards a responsive deep brain stimulation system for essential tremor

Authors: *E. OPRI[1], J. B. SHUTE[1], R. MOLINA[2], K. FOOTE[3], M. S. OKUN[4], A. GUNDUZ[1]; [1]Biomed. Engin., [2]Electrical Engin., [3]Dept. of Neurosurg., [4]Dept. of Neurol., Univ. of Florida, Gainesville, FL

Abstract: Essential Tremor (ET) is one of the most common movement disorders defined as a rhythmical, involuntary oscillatory movement of the limbs. Intention tremor occurs in the hands and arms, typically with a slow oscillation (~5-10 Hz). It is experienced during the initiation and execution of goal-directed reaching motions, while it is absent at rest. Although the pathophysiological basis of ET remains unknown, a pathological synchronous oscillation in a neuronal network involving the thalamus, especially the ventral intermediate nucleus (Vim), the premotor (PM) and primary motor (M1) cortices, and the cerebellum has been suggested. It is assumed that deep brain stimulation (DBS) suppresses tremor by masking the "tremor cells" in the Vim. The aim of this study is to implement a responsive therapeutic stimulation that would activate only in the presence of specific biomarkers such as movement intention (mu rhythm on motor and premotor cortices), presence of tremor (accelerometer), coherence between electrocorticography (ECoG) and accelerometer. This approach would allow theoretically to deliver an equally effective treatment while avoiding most of the stimulation side effects such as balance and speech impairment, and slowing the battery depletion of the implant.

Disclosures: E. Opri: None. **J.B. Shute:** None. **R. Molina:** None. **K. Foote:** None. **M.S. Okun:** None. **A. Gunduz:** None.

Poster

713. Brain Machine Interfaces: Invasive Applications

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 713.11/W33

Topic: D.18. Brain-Machine Interface

Title: Detection of tourette syndrome tics via centromedian thalamus lfp and acute trial of closed loop stimulation

Authors: *J. B. SHUTE[1], E. ORPI[2], R. MOLINA[2], J. ROSSI[3], M. OKUN[2], K. FOOTE[2], A. GUNDUZ[2];
[1]BioMedical Engin., UF, Gainesville, FL; [2]Univ. of Florida, Gainesville, FL; [3]Univ. of Florida, gainesville, FL

Abstract: Tourette Syndrome (TS) is a paroxysmal neuropsychiatric disorder characterized by involuntary movements or/or vocal outbursts known as tics. Deep brain stimulation (DBS) is an emerging therapy for cases of severe and intractable TS. DBS is an invasive neuromodulatory therapy in which depth electrodes are placed within deep subcortical structures of the brain and high frequency electrical stimulation is used to modulate pathological neural activity. Recent TS DBS studies have indicated that low frequency local field potentials between 1-15 Hz (lf-LFP) within the centromedian thalamus (CM) were observed during tics. We investigated the presence of lf-LFP in the CM. Two subjects, TS01 and TS02, suffering from severe and intractable TS were implanted with DBS. Both subjects were implanted with CM depth electrodes bilaterally and EcoG strips over the primary and premotor cortices (hand region). Subjects were asked to perform three conditions: Baseline (data containing no tics or movements), volitional movements (data containing no tics) and ticing (data containing tics but no volitional movements). LFP data was collected concurrently with accelerometers, EMG, and video. Data was collected from TS01 and TS02 during surgery and every month postoperatively for 6 months. A neurologist analyzed video data and tic times were determined. LFP data from the motor strip and CM was bandpassed into 10 Hz bins and rectified. The top 3 discriminated features (between baseline and tics) were used. A support vector machine was trained to detect tics. TS01 exhibited long duration tics that were dystonic in nature affecting primarily the right-sided arm, neck, and face. TS02 exhibited vocal tics, flicking the middle finger, and sustained mumbling tics. lfLFP were observed in both TS01 and TS02 at or around the time of tic. Tics of long duration (longer than 5 seconds) were detected with a high precision (85-100%) and recall (85-100%) across every month with TS01 and across the first 3 months with TS02. Short duration tics (less than 5 seconds) were detected with less than 70% recall and precision for every month. The top 3 features were low frequency CM (1-10Hz , 11-20Hz , 20-30Hz). No changes in CM LFP were observed during volitional movements or during baseline. lf-LFP were used to drive close loop stimulation in the acute setting. Stimulation was triggered off of positive detections and was verified with video and accelerometers.

Disclosures: J.B. Shute: None. **E. Orpi:** None. **R. Molina:** None. **J. Rossi:** None. **M. Okun:** None. **K. Foote:** None. **A. Gunduz:** None.

Poster

713. Brain Machine Interfaces: Invasive Applications

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 713.12/W34

Topic: D.18. Brain-Machine Interface

Support: T90 DA023436-02

NIH NS065186

NSF EEC-1028725

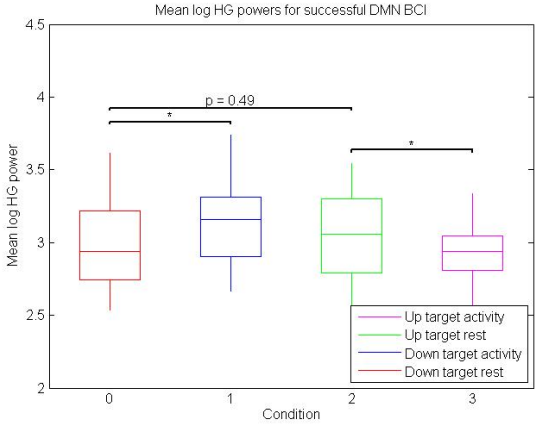
NIH 2K12HD001097

Title: Default mode network electrocorticographic brain-computer interface

Authors: *D. J. CALDWELL[1], J. D. WANDER[1], K. E. WEAVER[2], D. SARMA[1], J. D. OLSON[3], L. A. JOHNSON[4], R. P. N. RAO[5], J. G. OJEMANN[4];
[1]Ctr. for Sensorimotor Neural Engin. and Dept. of Bioengineering, [2]Ctr. for Sensorimotor Neural Engin. and Dept. of Radiology, [3]Ctr. for Sensorimotor Neural Engin. and Dept. of Rehabil. Med., [4]Ctr. for Sensorimotor Neural Engin. and Dept. of Neurolog. Surgery, [5]Ctr. for Sensorimotor Neural Engin. and Dept. of Computer Sci. and Engin., Univ. of Washington, Seattle, WA

Abstract: Objective: The use of brain-computer interfaces (BCIs), including those driven by electrocorticography (ECoG), allows for exploration of brain function exploration and rehabilitative applications. Commonly, BCIs are controlled by activity changes in primary motor cortex that occur when subjects perform motor imagery. The default mode network (DMN) comprises brain regions thought to be active during a resting state. During periods of attention, such as a BCI task, the neural activity in DMN regions *decreases*, which is referred to as task induced depression (TID). The goal of this study is to explore if TID can be used for BCI control. **Methods:** Subjects (n=3) implanted with ECoG grids performed a 2-target right justified box task (2-RJB) controlling the BCI with activity from an electrode over putative DMN regions. The electrode for control was picked as one showing high gamma (HG, 70-200 Hz) suppression during goal-oriented behavior. HG power increases drove the cursor down, and HG decreases the opposite. 2 subjects also attempted a 3-target RJB (3-RJB). **Results:** 1 of 3 subjects performed above chance for the 2-RJB. Neither of the 2 subjects performed above chance for the 3-RJB. Significant ($p<0.05$) differences in the HG power were seen between both up and down target presentation, when comparing neural activity during the task to a rest period for the above chance subject. For down targets, HG power increased relative to rest, while for up targets, HG power decreased. No significant difference was seen between the rest periods for up and down targets. **Conclusions:** Novel DMN BCI via ECoG derived signals was demonstrated in one subject. This subject modulated HG power both above and below baseline when completing the task, which is distinct from motor cortex-driven BCI, where modulation above baseline only is commonly observed. DMN electrode controlled BCI could be a viable alternative to motor

cortex control. However, as evidenced by the failure of 2 subjects to perform above chance, and the inability to execute the 3-RJB, DMN control may be more difficult and further development is necessary.



Disclosures: D.J. Caldwell: None. **J.D. Wander:** None. **K.E. Weaver:** None. **D. Sarma:** None. **J.D. Olson:** None. **L.A. Johnson:** None. **R.P.N. Rao:** None. **J.G. Ojemann:** None.

Poster

713. Brain Machine Interfaces: Invasive Applications

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Program#/Poster#: 713.13/W35

Topic: D.18. Brain-Machine Interface

Support: NIH Grant NS12542

NIH Grant NS40867

NIH Grant RR00166

W.M. Keck Foundation

Christopher & Dana Reeve Foundation

Japan Science and Technology Agency

Precursory Research for Embryonic Science and Technology

Title: Changes in post-synaptic efficacy of primate corticospinal cells is associated with compensatory changes in firing patterns

Authors: Y. NISHIMURA[1], S. I. PERLMUTTER[2], R. W. EATON[2], *E. E. FETZ[2];
[1]Dept. of Developmental Physiol., Natl. Inst. for Physiological Sci., Okazaki, Japan; [2]Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: Motor learning and functional recovery from brain damage involve changes in the strength of synaptic connections between neurons as well as changes in neuronal activity. Whether changes in post-synaptic efficacy of a neuron are reflected in its activity pattern is largely unknown. We previously documented that the size of post-spike effects of primate corticomotoneuronal (CM) cells can be modified with a recurrent neural interface that delivers spike-triggered stimuli in the spinal cord during free behavior (Nishimura et al., 2013). These post-spike effects on muscle activity are a direct measure of the strength of the cells' synaptic connections and were modified for particular spike-stimulus delays according to a spike-timing dependent plasticity rule. We also documented the directional tuning of these cortical neurons in a 2D target-tracking task requiring isometric forces about the wrist. Here, we show that cells with strengthened CS connection exhibited decreases in the tuning depth, indicating that stronger post-synaptic efficacy was associated with lower firing rates. Significant decreases in cell tuning were produced after conditioning with spike-stimulus delays between 12 and 25 ms, but not 50 ms and longer, consistent with the effects on synaptic strength. For some cells, the decreased cell tuning lasted for days after the end of conditioning, but in most cases tuning eventually reverted to preconditioning levels. This relation between changes in synaptic efficacy and changes in firing patterns indicates that firing rates compensate for the changed output of CM cells and synchronized cells.

Disclosures: Y. Nishimura: None. **S.I. Perlmutter:** None. **R.W. Eaton:** None. **E.E. Fetz:** None.

Poster

713. Brain Machine Interfaces: Invasive Applications

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 713.14/W36

Topic: D.18. Brain-Machine Interface

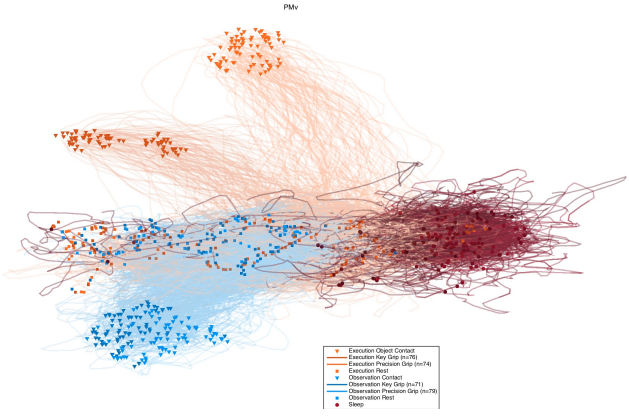
Support: NINDS-Javits (R01NS25074)

Katie Samson Foundation

Title: Neural ensemble activity characterizes sleep states, active movement, and movement observation in motor cortex

Authors: *J. B. ZIMMERMANN[1,2], C. E. VARGAS-IRWIN[1,2], J. P. DONOGHUE[1,2,3,4]; [1]Dept. of Neurosci., [2]Inst. for Brain Sci., [3]Sch. of Engin., Brown Univ., Providence, RI; [4]Ctr. for Neurorestoration and Neurotechnology, Rehab. R&D Service, Dept. of VA Med. Ctr., Providence, RI

Abstract: Detection of behavioral states is an important feature of any brain-computer interface suitable for 24h operation. Periods of sleep can resemble rest in local field potentials or movement when comparing firing rates. Here we demonstrate distinct representations of action, action observation, rest, and sleep periods in a low dimensional neural state space. We recorded simultaneous ensemble neural activity from primary motor cortex, and dorsal and ventral premotor areas from the rhesus macaque while the monkey was engaged in a reach to grasp task, observed an experimenter perform the same task, and during sleep. In accordance with previous experiments, we found that overall neural activity is decreased during sleep compared to movement, observation, and rest in all three areas. Nevertheless, short periods of high neural activity occur during sleep, with firing rates comparable to movement periods. We used spike train similarity measures and dimensionality reduction techniques to project ensemble activity during movement, action observation, rest, and sleep into low-dimensional neural state spaces. In all areas, active movement trials cluster separately from observation trials during object contact. Rest in inter-trial periods constitutes another cluster. Periods of sleep again constitute a separate cluster, with some overlap with resting periods. Whereas both action observation and movement trials exhibit a distinct pattern of cycles in neural state space, such organization does not seem to occur in sleep periods. Transient increases in firing rates occur during all the behaviors investigated, yet we can distinguish between different behavioral states using unique signatures in neural dynamics. Automatic detection and classification of such states will be a valuable tool for calibration of closed-loop BCIs.



Disclosures: **J.B. Zimmermann:** None. **C.E. Vargas-Irwin:** None. **J.P. Donoghue:** None.

Poster

713. Brain Machine Interfaces: Invasive Applications

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 713.15/W37

Topic: D.18. Brain-Machine Interface

Title: Predicting decision outcomes from single realizations of lateral prefrontal cortex neuronal activity

Authors: *C. BOULAY[1,3], M. LEAVITT[4], F. PIEPER[5], J. MARTINEZ-TRUJILLO[6,4], A. SACHS[2,3];
[1]Neurosciences, [2]Ottawa Hosp. Res. Inst., Ottawa, ON, Canada; [3]Univ. of Ottawa, Ottawa, ON, Canada; [4]McGill Univ., Montreal, QC, Canada; [5]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [6]Univ. of Western Ontario, London, ON, Canada

Abstract: Neurons in the lateral prefrontal cortex (LPFC) encode sensory and cognitive signals, as well as commands for goal directed actions. Thus, this brain region might be a good signal source for a goal-selection brain-computer interface (BCI) that decodes the intended goal of a motor action previous to its execution. Toward the development of a goal-selection BCI, we set out to determine if we could decode saccade goals from single-trial LPFC neuronal activity. We recorded neuronal spiking activity from microelectrode arrays implanted in area 8A of the LPFC of two adult macaques while they made visually guided saccades. The rewarded target was indicated by a colour cue and we changed periodically the association between colour and rewarded direction. We extracted neuronal firing from single-trial LPFC activity in the pre-saccade period and predicted saccade targets using support vector machines with 10-fold cross-validation. Binary decision outcomes were decoded from pre-saccadic single-trial LPFC activity with better-than-chance accuracy. Decoder performance remained high when examining groups of trials with identical visual stimuli but different behaviour (>80%). Further, some LPFC activity was dependent on contextual information independent of visual information and saccade performance. These results provide further evidence that LPFC neurons encode decision processes and suggest that LPFC activity can be used as a signal source for a goal-selection cognitive BCI.

Disclosures: **C. Boulay:** None. **M. Leavitt:** None. **F. Pieper:** None. **J. Martinez-Trujillo:** None. **A. Sachs:** None.

Poster

713. Brain Machine Interfaces: Invasive Applications

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 713.16/W38

Topic: D.18. Brain-Machine Interface

Support: JSPS KAKENHI grant Nos. 24243069

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JSPS KAKENHI grant Nos. 26115513

JSPS KAKENHI grant Nos. 24300148

JSPS KAKENHI grant Nos. 25560435

Title: Transfer of operantly conditioned firings between different neuron groups with BMI in rats

Authors: *K. SONG, S. TAKAHASHI, Y. SAKURAI;
Grad. school of brain science, Doshisha Univ., Kyoto-Hu, Japan

Abstract: It has been proved that controlling a neuroprosthesis is possible by selectively conditioned firings of a specific neuron group consisting of some neurons. It is also postulated that both a single neuron and a neuron group are able to be operantly conditioned to modulate their firings in isolation with neighboring neurons, which is called neural operant conditioning. However, it has not been clearly demonstrated if transfer of the operantly conditioned firings of a neurons group to other neuron groups is facilitated with neural operant conditioning. In this study, multiple neuron groups were recorded by multiple bundles of microwires implanted in the motor cortex of behaving rats. Experiment sessions started after firings of at least 2 neuron groups were identified in the rat's motor cortex. In pre-learning stage, the rats were trained to perform a behavioral free-operant task in which nose-poke behaviors were rewarded. During leaning stage, the rats were rewarded whenever firings of the neuron group 1 detected in real-time with a brain-machine interface (BMI) system satisfied preset criteria. After learning of the neural operant task with neuron group1 was completed, the rats conducted the identical neural operant task using firings of the neuron group 2. Data of each of the neuron groups were analyzed offline. Firing rates, reward rates and delays to reward delivery, were employed to examine the hypothesis that neuronal operant learning of neuron group 1 is transferred and facilitates the conditioned modulation of the neuron group 2. A preliminary results examining the hypothesis will be reported. It has been said that the most critical problem of neural operant conditioning using a small number of neurons is its limited life as sources of signals for

volitional motor outputs to control a neuroprosthesis. In this respect, transfer of operantly conditioned firings between different neuron groups is profitable to compensate the limited life of source signals. The result of this study is expected to contribute to advances in neurorehabilitation and neuroprosthesis.

Disclosures: **K. Song:** None. **S. Takahashi:** None. **Y. Sakurai:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.01/W39

Topic: E.05. Stress and the Brain

Support: FAPESP - 2014/22228-6

Title: Supraoptic nucleus of hypothalamus modulates cardiovascular responses evoked by acute restraint stress in rats

Authors: *S. LOPES AZEVEDO, E. A. FORTALEZA, A. A. SCOPINHO, C. BUSNARDO, F. M. A. CORREA;
Pharmacol., Sch. of Med. of Ribeirao Preto/USP, Ribeirão Preto, Brazil

Abstract: Objective: The present work studied the possible involvement of supraoptic nucleus of hypothalamus (SON) neurotransmission in the mediation of restraint stress-induced autonomic changes. Methods: Guide cannulae were implanted bilaterally in the SON of rats for the microinjection of either drugs or vehicle, and a polyethylene catheter was implanted into the femoral artery for recording of mean arterial pressure (MAP) and heart rate (HR) using a computerized acquisition system. Tail temperature was evaluated using a thermal camera. Rats were subjected to restraint stress 10 min after the microinjection of drugs or vehicle into the SON and studied the effect of the bilateral microinjection of the nonspecific synaptic blocker cobalt chloride (CoCl2, 1mM/100nL) into the SON on hypertension, tachycardia and a reduction in tail temperature induced by restraint stress. Results: The CoCl2 pretreatment of the SON significantly reduced the restraint-evoked increase in MAP ($F_{1,600}= 252.0$, $P<0.0001$, $n= 6$) and potentiated the restraint-evoked increase in HR ($F_{1,600}= 188.5$, $P<0.01$, $n= 6$), when compared with ACSF-treated animals (vehicle, 100nL, $n= 5$). Conclusion: These results show that local SON neurotransmission participates in the neural pathway which is involved in the modulation of pressor and tachycardiac responses observed during acute restraint stress in rats, suggesting

facilitatory and inhibitory roles respectively on MAP and HR of this structure in this aversive situation.

Disclosures: **S. Lopes Azevedo:** None. **E.A. Fortaleza:** None. **A.A. Scopinho:** None. **C. Busnardo:** None. **F.M.A. Correa:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.02/W40

Topic: E.05. Stress and the Brain

Support: DARPA #N66001-11-C-4006

Title: Heart-rate variability and power spectral densities as neurophysiological indices of post-traumatic stress disorder

Authors: ***R. T. CHANG**[1], S. J. SMITH[1], V. TAN[1], K. A. CORREA[1], M. CRYSTAL[2], R. JOHNSON[1], C. BERKA[1];
[1]Advanced Brain Monitoring, Carlsbad, CA; [2]BBN Raytheon, West Newton, MA

Abstract: The identification of the neurophysiological biomarkers for post-traumatic stress disorder (PTSD) has been an ongoing challenge for many researchers. Over the past decade, studies have examined the neurophysiology of PTSD using both electroencephalogram (EEG) and electrocardiogram (ECG) to investigate cognition and the autonomic nervous system (ANS). These studies have shown both EEG frequency bandwidths (BW) -particularly the Alpha and Theta range- and ANS activation, measured by low (LF) vs. high (HF) frequency ratio of heart rate variability (HRV), are altered in PTSD populations. Elevated LF:HF ratios are associated with greater sympathetic activation (and stress/anxiety states), while altered Alpha and Theta can be associated with arousal dysregulation. This study's objective was to further explore the neurophysiological biomarkers of stress by utilizing a tool that systematically elicits a genuine stress response while simultaneously recording EEG and ECG. A total of 96 participants from three different cohorts were recruited for the study: Healthy control, a high stress population measured by current level of major life stressors (MLS), and a population diagnosed with combat-related PTSD. A series of questionnaires and audio-visual stimuli were presented. The stimuli consisted of videos and images of generically negative content as well as negative content related to the participant's traumatic experience (e.g. autobiographical) indicated through their initial questionnaire. Initial results show that LF:HF ratios increased in all groups; with the

PTSD group showing a greater increase in sympathetic response from the baseline tasks (3CVT, A-PVT, and VPVT) to the audio-visual stimuli. In addition, there is an effect of task type (i.e. neutral vs. autobiographical content) for Theta (3-7Hz), Alpha (8-12Hz), Beta (21-30Hz), and Gamma (31-40Hz), indicating arousal changes throughout the protocol, across all conditions. These data suggest that those with PTSD are experiencing higher levels of arousal and stress that does not allow them to disengage from autobiographically reported stressors. Efforts to continue to build a foundation for the understanding of the biological basis and potential predispositions for PTSD may help enable the discovery of better interventions, training, and preventative solutions.

Disclosures: **R.T. Chang:** None. **S.J. Smith:** None. **V. Tan:** None. **K.A. Correa:** None. **M. Crystal:** None. **R. Johnson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock holder. **C. Berka:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); owner, stock holder.

Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.03/W41

Topic: E.05. Stress and the Brain

Support: KAKENHI 24102505

KAKENHI 24659024

Title: Sigma-1 receptor mediates depressive behaviors induced by cardiovascular diseases

Authors: *K. FUKUNAGA, Y. SHINODA;
Tohoku Univ. Grad Sch. Pharm Sci., Sendai, Japan

Abstract: Cardiovascular diseases are risk factor to cause the depression in human. We recently defined that an impaired sigma-1 receptor (Sig-1R) function accounts for SSRI-resistant depressive behaviors in CaMKIV null mice (Mol Neurobiol DOI 10.1007/s12035-014-8923-2). We here hypothesized that 1) the impairment of Sig-1R in brain triggers the depressive behaviors in cardiovascular disease patients and 2) Sig-1R stimulation improves its depressive behaviors. To test our hypothesis, cardiac hypertrophy and heart failure were induced by transverse aortic constriction (TAC). Hyperfunction of hypothalamo-pituitary-adrenal axis was induced by

chronic corticosterone administration in mice. Oral administration of a specific Sig-1R agonist SA4503 (0.3-1.0mg/kg) significantly improved TAC-induced depressive behaviors with restoration of Sig-1R expression in both hippocampal CA1 and DG regions. Indeed, the plasma corticosterone levels were significantly elevated in 6 weeks after TAC concomitant with depression-like behavior expression. The chronic corticosterone administration for 3 weeks caused depressive behaviors with the reduction of Sig-1R expression in the hippocampus. Consistent with the improvement of depression behaviors, heart failure was also ameliorated by SA4503 (0.3-1.0mg/kg) administration. Taken together, Sig-1R stimulation with SA4503 is attractive therapy to improve not only depressive behaviors but also heart failure following cardiovascular diseases. This work is supported by Grants-in-Aid for Scientific Research (Kakenhi 24659024 and 24102505 to K.F.) and Research Fellows of the Japan Society for the Promotion of Science (265570 to Y.S.)

Disclosures: K. Fukunaga: None. **Y. Shinoda:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.04/W42

Topic: E.05. Stress and the Brain

Support: PHC Volubilis MA/11/264 (24795PL)

GDRI Neuro

Title: Anorexia induced stress involved changes in neurons morphology and neurotrophic factors in the serotonin 4 receptors knockout mice

Authors: *M. EL OUAHLI[1], F. CHIGR[1], M. NAJIMI[1], V. COMPAN[2];
[1]Life Sci., Fac. of Sci. and Techniques, Beni Mellal, Morocco; [2]institut de Génomique Fonctionnelle, Montpellier, France

Abstract: Adaptive decision-making to eat is crucial for survival but in neurodegenerative and mental diseases, the brain persistently supports persistent changes in food intake. How the brain persists in reducing or enhancing food intake to the point of death despite the evolution of multiple mechanisms to ensure survival by governing adaptive eating behaviors in front of stress remains mysterious. In order to study the functions of 5-HTR4, we previously engineered the 5-HTR4 knockout mice (KO4) and evidenced their critical role in anorexia-like and binge eating.

Here, we show an absence of increase in the production of the phosphorylated cAMP responsive element binding protein (CREB) in the hypothalamus in KO4 mice following acute stress. As pCREB is a transcription factor, we conducted transcriptom and RQ-PCR analyses. We evidenced that the 5-HTR4 control the expression of neurogenesis and DNA methylation factors suggesting that 5-HTR4 could induce persistent morphological changes of neurons and favor a persistent restrictive food intake. Consistently, we found that 5-HTR4 exert a positive control of the anorectic brain-derived neurotrophic factor in the medial prefrontal cortex, in which 5-HTR4 control stress-induced anorexia. The number of dendritic spines of pyramidal cells in the medial prefrontal cortex is reduced in the absence of 5-HTR4, which may support the abnormal resistance of KO4 mice to chronic stress-induced hypophagia. The present study shows that 5-HTR4 may contribute to implement neural networks by controlling gene expression of neural growth factors that are involved in adaptive behavior to stress.

Disclosures: **M. El Ouahli:** None. **F. Chigr:** None. **M. Najimi:** None. **V. Compan:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

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Program#/Poster#: 714.05/W43

Topic: E.05. Stress and the Brain

Support: GDRI Neuro CNRS/CNRST

PICS CNRS/CNRST

Neuromed FP7

Title: Stress, anorexia and neuroendocrine regulation of food intake

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Abstract: Food intake (FI) regulation in adult mammals is integrated mainly by 2 brain structures: the hypothalamus and the dorsal vagal complex (DVC). Short-term regulation, which consists in reflex arrest of FI under stomach filling or satiety reflex, is triggered by vagus nerve afferents to the DVC. Long-term regulation consists essentially in satiety reflex threshold which involve the hypothalamus and its reciprocal connections with the DVC Furthermore, numerous circulating peptides influence FI through their actions on the hypothalamus, the brainstem and the autonomic nervous system. The physiological importance of this homeostatic control system

is highlighted by FI deregulations causing eating disorders like anorexia and their associated complications. The relation between several stress paradigms and FI dysregulation is well evidenced. They may affect the central regulation, probably by interacting with factors involved in this regulation. Therefore, we decided to study the long-term effects of a single exposure to immobilization stress (IS)on the expression of anorexigenic and orexigenic factors such as neuropeptide Y (NPY), Agouti Related Peptide (AgRP), Pro-opiomelanocortin (POMC) and cocaine- and amphetamine- regulated transcript (CART)in different brain structures related to FI control. In order to investigate the involvement of the signaling of these peptides in the food control, notably in the context of FI alterations (anorexia caused by stress), we analyzed the hypothalamic and DVC expression of the mRNAs of these peptides. We showed, by using RT-PCR that the mRNAs of the peptides analyzed display significant increases in stressed rats compared to controls, although with differential peaks. In hypothalamus, NPY and CART transcript up-regulation is observed at the end of IS and persists until 48-72h after IS. In the DVC, expression of the 2 transcripts peaks significantly at 24h post-stress and decline afterwards; NPY mRNA remains then significantly higher than in controls, whereas CART mRNA is down-regulated after 48h post-stress. The comparison between the expression profiles of anorexigenic and orexigenic peptides investigated shows also the presence of a parallelism between that of POMC and AgRP. The persistence of alteration of the expression of anorexigenic and orexigenic factors during the post-stress period could be highly related to the slow recovery of the hypothalamo-hypophyseo-adrenal (HPA) axis in IS and points to stress-induced plasticity in both nervous centers of food intake regulation.

Disclosures: **M. Najimi:** None. **F. Chigr:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.06/W44

Topic: E.05. Stress and the Brain

Support: JSPS KAKENHI 23228004

JSPS KAKENHI 25292185

Title: Anti-inflammatory effects of PGRN on the temperature and food intake via suppressing the level of circulating cytokines

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The Univ. of Tokyo, Tokyo, Japan

Abstract: Progranulin (PGRN) is a multifunctional growth factor expressing widely in the whole body. We have previously identified PGRN as one of the genes that play important roles in masculinization of the brain during the perinatal period. On the other hand, it has recently reported that haploinsufficiency of PGRN is the major factor causing frontotemporal lobar degeneration. Subsequently, many other researches including ours have demonstrated the neuroprotective, neurotrophic functions of PGRN. We have also shown that PGRN is involved in voluntary exercise-induced neurogenesis and suppression of neuroinflammation after traumatic brain injury. As PGRN is expressed in the immune cells both in the peripheral and central tissues, the main purpose of the present study is to elucidate the role of PGRN in the inflammatory responses to immune challenge. In all the experiments, male C57BL/6J wild-type (WT) mice or PGRN-deficient (KO) mice of the same background were used. We intraperitoneally injected lipopolysaccharide (LPS, 120 μg/kg) to the animals and measured the body temperature for 9 hours in the daytime and food intake for 24 hours. Although LPS induced fever response and anorexia in the mice of both genotypes, the symptoms were much severer in the KO mice. LPS is known to induce the secretion of the inflammatory cytokines that transmit the immune signal from the peripheral to the central tissues. Thus, we subsequently determined the serum concentration of inflammatory cytokines i.e. IL-1β, IL-6 and TNF-α at 0, 1 and 3 hours after LPS injection. KO mice showed the significantly stronger induction of the IL-6 at 3 hours and TNF-α at both 1 and 3 hours after injection. IL-1β also had the tendency of stronger induction at 3 hours in KO mice although it did not reach statistic significance. These results suggested that PGRN suppresses excessive inflammatory responses by moderating secretion of inflammatory cytokines in the peripheral tissue.

Disclosures: **T. Matsuwaki:** None. **K. Yamanouchi:** None. **M. Nishihara:** None.

Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: NIDDK-NIH Intramural Research Grant

Title: Erythropoietin and high fat diet-induced brain inflammation

Authors: S. DEY[1], J. ANHUT[1], M. GASSMANN[2], *C. T. NOGUCHI[1];
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Abstract: Erythropoietin (Epo), known for its hematopoietic role, inhibits obesity-induced white fat inflammation in mice, is also produced in brain by astrocytes, and its receptor (EpoR) is expressed in microglial cells. Microglial cells respond to saturated fatty acids present in high fat-diet (HFD) by producing inflammatory cytokines that disrupt the energy homeostatic regulation by the hypothalamus. We hypothesized that Epo signaling in brain could regulate HFD-induced microglial activation and thereby maintain metabolic homeostasis. Intracerebroventricular Epo (ICV-Epo) administration in HFD-fed wild-type mice for 4 weeks resulted in lower fasting glucose levels and lower weight gain compared to saline control (ICV-saline). Moreover, ICV-Epo and ICV-saline groups did not show any difference in hematocrit, indicating no peripheral hematopoietic effect, and showed equal food intake during that time period. ICV-Epo administration significantly reduced brain mRNA expression of inflammatory markers IL-1β, TNFα, and SOCS3 and increased mRNA levels of anti-inflammatory protein IL-10 compared to saline control. Chronic over-expression of human Epo transgene in brain (Tg21 mouse model) also prevented increase in fat mass after HFD-feeding for 3 weeks compared to wild-type (WT) control. We examined primary mouse microglial cells to assess direct Epo anti-inflammatory response using palmitic acid (PA) that activates inflammatory response. PA significantly increased TNF-α, IL-1β, and SOCS3 mRNA, while co-treatment with Epo significantly reduced TNF-α and IL-1β mRNA expression and showed an increased trend in IL-10 mRNA expression. In summary, these studies suggest an important extrahematopoietic and homeostatic function of Epo in brain inflammation and metabolism.

Disclosures: S. Dey: None. **J. Anhut:** None. **M. Gassmann:** None. **C.T. Noguchi:** None.

Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: NIMH Grant R01MH097676

VA Research Merit Award BX000935

Title: T cells mediate behavioral, hormonal and cytokine responses in the learned helplessness paradigm

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Abstract: Accumulating evidence supports a multifaceted role for T cells in behavioral responses to stress. Whether this role is protective against or potentiates the detrimental effects of stress appears to depend on the type of T cell studied, the mode and intensity of stressor employed, and the genetic background of the animal model utilized. In this study we examined the impact of naïve $CD4^+$ and $CD8^+$ T cells on several aspects of the stress response in immune deficient *Rag2*$^{-/-}$ and wild type (WT) C57BL/6 mice that underwent the learned helplessness (LH) paradigm. This protocol allows for the evaluation of these responses immediately following an initial, inescapable stress session and after repeated stress exposure. *Rag2*$^{-/-}$ mice in this study were more resilient in the LH paradigm, displaying reduced behavioral interference and a more rapid recovery to basal plasma corticosterone levels compared to WT mice. While reconstitution with T cells failed to completely recapitulate the WT behavioral phenotype there was a shift towards increased susceptibility and a significant increase in plasma corticosterone in the absence of overt T cell activation. Furthermore, the presence of T cells was sufficient to reduce levels of mature brain derived neurotrophic factor in the hippocampus after inescapable stress exposure, suggesting that T cell mediated regulation of neurotrophic factors may be an important link between the immune system and changes in neuronal function and subsequently, behavior. Our results indicate that T cells, are capable of affecting several facets of adaptive responses to stress, including behavioral, hormonal and cytokine responses.

Disclosures: **S.M. Clark:** None. **C. Song:** None. **X. Li:** None. **L.H. Tonelli:** None.

Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: CONACyT CB2014 No.243419

FIS/IMSS/PROT/G14/1299

Title: Early life stress activates microglial cells and induces an inflammatory response in the hippocampus of male rat pups

Authors: *L. TORNER[1], A. ROQUE GALICIA[1], A. OCHOA ZARZOSA[2];
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Abstract: Adult animals subjected to chronic stress show an inflammatory response in the hippocampus which has been related to cognitive dysfunction and psychopathology. However the immediate consequences of early life stress on hippocampal glial cells have not been studied. Here we analyzed the effects of maternal separation (MS) on astrocyte and microglial cell morphology in the hippocampal hilus, compared the expression of cytokines in the hippocampus and hypothalamus, and the peripheral response of cytokines, under basal conditions or stress exposure, on postnatal day (PD) 15. Male rat pups of MS (3h/day, PD1-PD14) and Control (undisturbed) groups showed similar cell densities of microglial cells in the hilus, but MS pups had significantly more activated microglia. The number of astrocytes was decreased and had fewer branches in the MS group compared to control group. Cytokine mRNA expression (qPCR) was analyzed in MS and Control groups, sacrificed i) under non-stressed, basal (B) conditions or ii) after a single stress event (SS) at PN15. Hippocampal extracts from MS pups showed an increase in IL-1β mRNA, under basal (B) and after a single stress (SS) event, compared to Controls. IL-6 was unchanged in both groups under B and SS conditions. TNF-α mRNA increased in control pups only under SS conditions. In MS pups, TNF-α mRNA increased in B but not after SS. In hypothalamic tissue, no differences were found in cytokine expression levels between both groups. In trunk blood, MS decreased IL-1β levels under B and SS conditions; IL-6 levels decreased in B but increased after SS in MS pups. TNF-α levels were unchanged in both groups. In conclusion MS activates microglial cells, decreases astrocyte number, and exacerbates the immediate inflammatory response to stress in the hippocampus, but triggers a differential response of peripheral cytokines. This indicates an independent response to stress of the brain immune system to that of the periphery. (Supported by CONACyT CB2014 No.243419 and FIS/IMSS/PROT/G14/1299)

Disclosures: **L. Torner:** None. **A. Roque Galicia:** None. **A. Ochoa Zarzosa:** None.

Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: Indiana University

Title: Differential effects of stress on microglial cell activation in male and female medial prefrontal cortex

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Abstract: Susceptibility to multiple stress-linked psychological disorders differs between men and women, with women nearly twice as likely to suffer from post-traumatic stress disorder and major depression. Various stress-linked structural and functional changes in medial prefrontal cortex have been implicated in these disorders, alongside numerous cognitive-behavioral deficits. Chronic stress affects medial prefrontal cortex in a sex-dependent manner, differentially remodeling neuronal morphology and disrupting prefrontally-mediated behaviors in males and females. Emerging evidence suggests that acute and chronic stress induce microglial cell-mediated inflammation in medial prefrontal cortex. In male rats, chronic restraint stress increases microglial cell density and activity, reflected in altered microglial morphology in medial prefrontal cortex. Unstressed female rats exhibit increased microglial cell density and ramification in several brain regions compared to males, suggesting both heightened basal activation and a potential for sex differences in the effects of stress on microglial cell morphology. Therefore, we assessed microglial cell density and morphology in the prelimbic region of medial prefrontal cortex in male and female rats following acute (3 h, 1 d) or chronic restraint stress (3 h/d, 10 d). Control animals were left unhandled except for weighing. On the final day of restraint, rats were euthanized and brains were processed for visualization of microglia via Iba-1 immunohistochemistry. Microglia in medial prefrontal cortex were classified as ramified, primed, reactive, or amoeboid, and counted stereologically. Unstressed females showed a greater proportion of primed microglia relative to males. Acute and chronic restraint stress reduced the proportion of primed to ramified microglia in females, but did not significantly alter microglial morphology in males. This sex difference in microglial activation could contribute to the differential effects of stress on medial prefrontal cortex structure and function in males versus females.

Disclosures: J.L. Bollinger: None. **C.L. Wellman:** None.

Poster

714. Somatic Correlates of Stress

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Support: JSPS KAKENHI Grant Number 23228004

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International Research Fellow of the JSPS

Title: Involvement of progranulin in regulating neurogenesis and microglial activation in the hippocampus under acute infectious stress conditions

Authors: *Y. MA, T. MATSUWAKI, K. YAMANOUCHI, M. NISHIHARA;
The Univ. of Tokyo, Tokyo, Japan

Abstract: Infectious stress stimuli have been shown to suppress adult neurogenesis and enhance microglial activation in the hippocampus. We have previously suggested that progranulin (PGRN), a multifunctional growth factor, mediates the stimulatory effect of voluntary exercise on adult neurogenesis. In addition, PGRN also plays a suppressive role in neuroinflammatory responses related to activated microglia after traumatic brain injury. Thus, in the present study, we investigated the possible involvement of PGRN in the suppression of hippocampal neurogenesis and microglial activation under infectious stress conditions, i.e., low- or high-dose challenge of lipopolysaccharide (LPS). Twenty-two hours after LPS treatment, mice were intraperitoneally injected with bromodeoxyuridine (BrdU) to label proliferating cells. Two hours after BrdU injection, their brains were processed for immunohistochemical staining for Ki67 as well as BrdU. The results showed that the expression of PGRN mRNA in the hippocampus was significantly increased 24 h after high-dose LPS injection, while low-dose LPS did not affect PGRN mRNA levels at any time point. Compared with the vehicle-injected animals, the numbers of Ki67- and BrdU-positive cells in the subgranular zone (SGZ) of the dentate gyrus (DG) were significantly decreased in both low- and high-dose LPS-injected animals. However, the numbers of Ki67- and BrdU-positive cells in the SGZ were not significantly different between wild-type (WT) and PGRN-deficient (KO) mice injected with either low- or high-dose of LPS. The Iba1- and CD68-positive areas in the DG in the low- and high-dose LPS-injected mice were significantly larger than those in the vehicle-injected animals. Although the Iba1-positive areas were not significantly different between WT and KO mice following either low- or high-dose LPS stimulus, high-dose LPS significantly enhanced the CD68-positive areas in the DG in KO mice. By contrast, low-dose LPS did not affect the CD68-positive areas between WT and KO mice. These results suggest that PGRN is strongly linked to the down-regulation of microglial activation, while PGRN may not be involved in protecting hippocampal neurogenesis, under acute infectious stress conditions.

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Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: NIH R01 DA026597

Title: Gene network analysis of spinal cord in the model of chronic water avoidance stress

Authors: *S. BRADESI[1], I. KARAGIANNIDIS[2], K. BAKIRTZI[2], S. MAHURKAR JOSHI[3], D. ILIOPOULOS[3], C. POTHOULAKIS[2], E. A. MAYER[1];
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Abstract: Certain types of stress can have harmful effects at the cellular level inducing damage to proteins, mRNA or DNA. In rats, chronic water avoidance stress (WAS) is associated with changes in the expression of specific glial markers in the spinal cord. We aimed to test the hypothesis that WAS induces change in protein encoding gene and microRNA (miRNA) expression, which may have a significant role in the neuroimmunomodulation of spinal nociception signaling. Methods: Male Wistar rats were exposed to a daily session of WAS for 10 days, or handled daily. At day 11, L6/S1 spinal segments were collected and processed immediately for mRNA and miRNA isolation followed by gene expression profiling using the Agilent SurePrint G3 Rat Exon Microarray and Rat miRNA Microarray platforms. Lists of differentially expressed genes were generated using the dChip software program. Microarray analysis was performed using the Ingenuity Pathways Analysis (IPA) tool from Ingenuity Systems. Quantitative real time RT-PCR was performed using a 7500 Fast Real-Time PCR sequence detection system. Results: IPA identified 70 genes affected by WAS (FDR<0.05) belonging to pathways which include IL-6 signaling, PI3K/AKT and acute phase response signaling. A list of 39 miRNAs, part of two networks with relevance to inflammatory processes, showed differential expression in WAS compared to control rats. An analysis of the miRNA predicted targets in our 70 gene list showed the following target genes: gp130, I kappa b kinase epsilon, and Hsp40 homolog, subfamily C, member 21 (DNAJC21) as potential targets for miR-148-3p, miR17-5p, miR181a-5p, miR19b-3p and miR24-3p. We verified increased expression of mir17-5p and miR19b-3p in stress using qPCR. In addition, we observed changes in the expression of gp130 (essential in signal transduction for the family of IL-6-type cytokines) and STAT3 (involved in intracellular signaling cascades in response to gp-130 activation), both

predicted targets for mir17-5p. A reduced GFAP gene expression was also found in stress. Conclusion: Using a high throughput method, we demonstrated the effect of WAS on several networks involved in inflammatory pathways, which are relevant to pain signaling modulation. Our findings, suggesting a link between mir17-5p upregulation and change in gene expression of gp130 and STAT3 (known to affect GFAP gene transcription), provide new insight into the possible mechanisms mediating the effect of chronic stress on neuroinflammation in the spinal cord.

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Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: ONR Grant N00014-14-1-0787

CIHR Grant GSM-136180

Title: Structural and functional consequences of chronic psychosocial stress on the microbiota-gut-brain axis and immunity

Authors: *A. BHARWANI[1], J. BIENENSTOCK[1], J. FOSTER[2], M. SURETTE[3], P. FORSYTHE[3];
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Abstract: The gut microbiota and the brain are engaged in persistent bidirectional interplay--a phenomenon that influences host neural function and behaviour. However, the functional relationship between the microbiota and stress-induced changes in brain and behaviour, and underlying pathways of communication, remain unknown. To elucidate this relationship, we profiled the intestinal microbiome, behaviour, and immune function of mice exposed to psychosocial stress. We also investigated the potential of microbe-based strategies to modulate such changes, through chronic administration of *Lactobacillus rhamnosus* (JB-1). Male C57BL/6 mice subjected to chronic social defeat for ten days were assessed for changes in social, anxiety-like, and exploratory behaviours. Genomic DNA was isolated from fecal samples to characterize

the microbiome profile. 16S rRNA-derived operational taxonomic units (OTUs) were used to predict the functional metagenomic content of the microbiota. To investigate gut-brain signaling, splenocytes were analyzed for changes in the immune cell population using flow cytometry. Serum and supernatant from stimulated splenocytes were assessed for functional changes in the release of soluble signals. In a preliminary study, mice were orally administered $1.67x10^9$ CFU of *L. rhamnosus* (JB-1) for 28 days, leading into the defeat procedure. Stress-exposure altered the overall microbiome composition, and reduced species richness and diversity. Predictive metagenomic analysis suggested functional changes in the microbiome following stress exposure, including reduced functional diversity as well as the down-regulation of pathways involved in the synthesis and metabolism of neurotransmitter precursors and fatty acids. Defeated mice exhibited reduced social and exploratory behaviours, and lasting changes in the immune profile, including increased dendritic cell maturation and activation, reduction in the population of CD4+ CD25+ IL-10+ regulatory T cells, and increased serum levels of IL-6, but not TNF α. Preliminary results suggest that *L. rhamnosus* administration attenuated stress-induced deficits in sociability and exploratory behaviour, but not aggressor interaction. These findings demonstrate an association between stress-induced behavioural deficits and structural and functional changes in the microbiome. The study also identifies immune changes as a potential cause or consequence of microbiota-gut-brain communication.

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Poster

714. Somatic Correlates of Stress

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Grants-in-Aid for Scientific Research No. 25463010 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan

Title: Stress-induced galectin-1 in serum accumulates in lymphoid organs and may modulate the immune response

Authors: *T. KADOYA[1], K. YAMADA[2], Y. NARIMATSU[2], M. OONUKI[2], T. YAMAMOTO[2], K. KODA[1], K. SASAGURI[3];
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Abstract: We demonstrated that restraint stress increased the level of Galectin-1 (Gal-1) in the serum and the increase was regulated by the sympathetic nervous system but not the hypothalamic-pituitary-adrenal axis. Gal-1 is a widely expressed animal lectin with an affinity for β-galactoside and shows a variety of biological activities, such as immune-cell homeostasis, nerve regeneration after injury and the inflammatory response. These results suggest that the stress-induced serum Gal-1 may play an important role in preventing physiological and/or psychological stress through the sympathetic nervous system. In this study, we investigated the levels and the localization of Gal-1 in lymphoid organs, thymus and spleen, at 30min and 60 min after the restraint stress. Western analysis demonstrated the Gal-1 level in both organs of the stress group significantly increased compared to the controls. On the other hand, RT-PCR analysis indicated that the Gal-1 mRNA level did not increase after the stress. Immunohistochemical analysis showed that the immunoreactivity in the periarterial lymphatic sheath (PALS) of the thymus was stronger than in red and white pulp. In the spleen, Gal-1 staining was higher in the medulla than in the cortex. Furthermore, the Gal-1 co-localized with CD45+ lymphocytes in the PALS and the medulla. Thus, these results suggest that stress-induced Gal-1 in serum immediately accumulates in immune organs and may modulate the immune response through apoptosis by binding to CD45+ lymphocytes following physiological and/or psychological stress.

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Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: CONACyT grants No. 138663

CONACyT grants No. 129303

Title: Biochemical analysis of markers of oxidative stress and its association with psychological stress in students

Authors: *A. E. GONZÁLEZ, SR[1], S. GONZÁLEZ[2], E. BALTAZAR-GAYTAN[2,4], P. AGUILAR[3], G. FLORES[2];
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Abstract: In these days, the accelerated lifestyle of people has become one of the most important factors in the generation of mental disorders. Depression in young people can lead to the development of some serious problems like deficiency in school performance, substances abuse, violence, anxiety, eating disorders and suicidal ideation. There is evidence linking stress with the development of many psychiatric disorders. The body's response to a stressful stimulus is performed by the activation of the stress system, which causes changes in a large number of cells, increasing their metabolism, and free radicals production. When the generation of free radicals exceeds the antioxidant capacity of the organism, conduce to a harmful state, called oxidative stress, and begins damaging many vital structures for cell survival, such as DNA or cell membranes. This study aims to describe the relationship between oxidative stress and psychological stress caused by an academical test. For this, we analyzed the variation in the concentration of nitric oxide (NO) and Zinc (Zn), and the activity of the superoxide dismutase enzyme (SOD) in serum of 88 students from the Faculty of Chemistry Sciences of the Autonomous University of Puebla, with a range of ages from 19-24 years. The study was conducted in 3 groups of students taking classes with the same teacher (n1 = 32, n2 = 37, n3 = 19). The results show that there is indeed a variation in the concentrations of NO, Zn and SOD in serum samples taken one week before, during and one week after the test. The values of NO and SOD vary in proportion to the psychological demand caused by the stressor; however, serum Zn levels inversely changes due to the intracellular requirement during stress. The analysis of the variation in serum concentrations of NO and Zn, are good markers for determining the state of oxidative stress caused by an exam. The enzymatic activity of SOD however, doesn't show evident results with this stress level. (Supported by: CONACyT grants No. 138663 and 129303 to G Flores).

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Poster

714. Somatic Correlates of Stress

Location: Hall A

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Program#/Poster#: 714.16/X6

Topic: E.05. Stress and the Brain

Title: Suggestion of the analysis method to extract the relationship between the brain activity and salivary metabolites during acute psychosocial stress

Authors: *T. OKAMURA, T. HIROYASU;
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Abstract: [Objective] In recent years, along with the development of the nerve imaging technology and the metabolome analysis technique, the kinds of data which are extracted from the living body increased, and the numbers became enormous. Therefore analysis method to extract the relationship of these data is required. In this study, suggestion of the method to extract the salivary metabolites related to a brain activity state is performed. As an evaluation experiment, brain activity and the salivary metabolites during acute psychosocial stress are measured, and a relationship between brain activity and the salivary metabolites are investigated using the proposed method. [Proposed method] The proposed method is summarized as follows. A target task is set, and the brain activity is measured using fMRI (functional Magnetic Resonance Imaging). Through the experiment, saliva samples are gathered to investigate metabolic information. Using brain activity information of plural subjects, the brain activity state is performed clustering and become the label. The brain activity information of each subject has each label. Saliva samples are analyzed using capillary electrophoresis-MS and density of salivary metabolites is acquired. The density of metabolites is the feature value. The discriminator which can classify the above-mentioned label is learned. SVM (Support Vector Machine) is employed to learning. Leave-one-out cross-validation is performed for all subjects, and a mean identification rate is obtained. The combination of analyzed metabolites which maximizes the mean identification rate is obtained using GA (genetic algorithm). It is found that the combination of metabolites provided in this way is metabolites related to the brain activity state for the target task. [Experimental method] A psychosocial stress experiment is carried out to verify the validity of the proposed method. Eight healthy young men (22±1 years old) took part in this study. Psychological stress was induced using the Montreal Imaging Stress Task (MIST), where subjects are exposed to challenging mental arithmetic presented on a computer screen. Eight saliva samples were acquired, starting 35 min before the onset of the MIST until 45 min after the MIST.

Disclosures: **T. Okamura:** None. **T. Hiroyasu:** None.

Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: NSF Grant 0822129

Title: Habituation of plasma corticosterone and neuroimmune alterations in response to repeated daily exposure to several distinct stress challenges in Sprague Dawley rats

Authors: *D. LOVELOCK, M. SAMMAKIA, T. DEAK;
Behavioral Neurosci. Program, Dept. of Psychology, Binghamton Univ., Binghamton, NY

Abstract: Exposure to stress involves activation of the HPA axis, synthesis of proinflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), and activation of microglia. These experiments explored how neuroimmune and neuroendocrine responses to an acute stress challenge (footshock) would be altered by prior exposure to daily chronic stress, termed chronic escalating stress (CES). This model utilized a combination of established procedures that involved exposure to predictable daily challenges, yet introduced a systematic escalation in both the intensity and length of daily stress challenges. Adult male Sprague-Dawley rats were exposed to an 11 day procedure, where days 1-5 consisted of 60 min of restraint, days 6-10 consisted of 60 min of restraint immediately followed by 30 min of forced swim, and on day 11 subjects were exposed to an 80 shock, 2 hour intermittent footshock challenge. Experiment 1 examined adaptation in the corticosterone (CORT) response at key points in the procedure. We found that habituation to restraint occurred by day 6, and that the escalation in stressors from day 6 to 10 partially disrupted the established habituation, suggesting that restraint likely became predictive of imminent swim stress. The response at the timepoint following swim increased across experiment days 6 and 10, and the response to 2 hours of intermittent footshock did not differ between controls and the CES group. Experiment 2 investigated the impact of this stress paradigm on the expression of several important cytokine (IL-1, IL-6, TNF-α) and cellular activation markers (c-Fos, CD14, CD200R) in key brain regions (paraventricular nucleus of the hypothalamus, hippocampus, & prefrontal cortex). As expected, acute footshock exposure led to expected increases in c-Fos in all brain regions, increased IL-1 in the PVN, and suppressed TNF-α in the HPC and PFC. These changes were largely unaffected by prior exposure to CES paradigm, with mildly increased c-Fos expression in the HPC and PFC being the only differences of note between the acute footshock and CES groups. Thus, recent stress history did not modulate cytokine gene expression, indicating that the key driver of cytokine changes was the final footshock challenge, not a history of chronic stress. Additionally, the CES model induced an interesting dissociation between adaptation in the HPA axis and the neuroimmune response, where it had clear effects on CORT habituation but the acute

neuroimmune response was unaffected. This CES model may serve as a highly tractable model for studying adaptation to chronic stress relative to previously established paradigms.

Disclosures: **D. Lovelock:** None. **M. Sammakia:** None. **T. Deak:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.18/X8

Topic: E.05. Stress and the Brain

Support: NIH Grant R21MH101663

Title: Implications of stress and infection on neuroimmune function and behavior as potential risk factors for developing postpartum depression

Authors: *C. K. POSILLICO, J. M. SCHWARZ;
Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Drastic changes must take place in the function of the maternal immune system to foster the development of a growing fetus and to prevent it from being "attacked". Changes in immune function may also explain why women suffering from autoimmune diseases find improvement of their symptoms during pregnancy. Furthermore, it is well-known that neuroimmune changes have been linked to several psychiatric disorders including depression. Despite evidence of peripheral immune changes induced by pregnancy, no one has ever examined whether pregnancy or parturition induces changes in the central immune system simultaneously and whether this may be an underlying risk factor for postpartum depression. Therefore, we hypothesize that changes in immune function associated with pregnancy and parturition confounded with external stressors or infection may be linked to an increased risk for developing postpartum depression. Microglia are the immune cells of the brain, and they have both pro- and anti-inflammatory responses to various insults. Using real-time PCR, we identified significant changes in microglial activation markers and pro-inflammatory molecules within the medial prefrontal cortex (mPFC) and the hippocampus of pregnant and postpartum rats compared to non-pregnant controls. Females exposed to forced swimming stress during their last week of gestation showed a significant decrease in the expression of pro-inflammatory cytokine IL-1β in the mPFC as a result of the stress. Additionally, the experience of pregnancy or parturition in these animals further decreased IL-1β expression and increased IL-6 expression. Stress and pregnancy significantly decreased sucrose preference scores immediately postpartum;

however, the effects of both factors were not additive, and the effects were no longer seen one week postpartum. In a separate cohort of animals, females were given an injection of lipopolysaccharide (LPS, 100 μg/kg) one day prior to parturition. Similar to the previous experiment, pregnancy and immune activation increased the expression of IL-6 in the mPFC; however, the effects of both factors were not additive. Thus, these data indicate that pregnancy alone induces neuroimmune and behavioral changes. Additionally, stress or infection during late gestation interact with pregnancy resulting in unique neuroimmune gene expression and immediate depressive behavioral outcomes.

Disclosures: C.K. Posillico: None. **J.M. Schwarz:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.19/X9

Topic: E.05. Stress and the Brain

Support: NIH Grant DE021888

Title: Role of c-Src in oxidant-mediated Toll-like receptor 4 signaling

Authors: *O. J. IGWE, Y. ZHANG, R. KARKI;
Div. of Pharmacol. & Toxicology, Univ. Missouri- KC, Kansas City, MO

Abstract: Background: Oxidative stress is implicated in the initiation and progression of many diseases. ROS can act as second messengers in intracellular signaling to induce a dysregulated phenotype. But changes in cell signaling that may result in decreased or enhanced responsiveness after exposure to exogenous oxidants is not understood. Protein tyrosine kinases and phosphatases play a role in oxidant-induced cell signaling. As a leading member of the Src family of non-receptor tyrosine kinases, c-Src is involved in diverse signaling pathways. As most of c-Src is in a dormant state under basal conditions, regulation of its activation is crucial for its biological functions. c-Src is sensitive to cellular redox stress, but its role in oxidant-induced inflammation is not clear. Oxidants can activate NFB through stimulation of TLR4, which plays a critical role in innate and adaptive immunity. Here, we sought to establish a primary role for c-Src in oxidant-induced TLR4 signaling through the NFB activation. **Objective:** The purpose of the present study was to determine the role of c-Src in the molecular mechanisms that may underlie oxidant-induced NF-B activation through TLR4 signaling. **Methods:** We used HEK-Blue cells that are stably transfected with mouse TLR4 and that express secreted embryonic

alkaline phosphatase (SEAP) reporter gene under the control of a promoter inducible by NF-B. We used LPS and MPLA as TLR4 specific agonist and positive controls, and SIN-1 and PPC as sources of oxidants. The level of SEAP released due to TLR4 stimulation was a measure of NF-B activation. We used fluorescence imaging to measure the levels of intracellular ROS (iROS) after oxidant treatment. We quantified oxidant-induced lipid peroxidation as thiobarbituric acid reacting substances. We determined the activity of c-Src and the product of ROS-induced NF-B activation by ELISA and immunoprecipitation, respectively. **Results:** Treatment with either the oxidants or LPS-EK increased iROS with an enhanced production of nitric oxide and TBARS to confirm oxidative stress, SEAP release and TNF- production. Pretreatment with c-Src inhibitors, PP2 and CA-pY, which act by different mechanisms, decreased these parameters. Pretreatment with SSG, a c-Src activator, enhanced the effects promoted by LPS-EK and oxidants, and rescued cells from PP2- and Ca-pY-induced effects. Both oxidants and TLR4 agonist increased formation of c-Src complexes with TLR4 and IB- as coimmunoprecipitates. **Conclusion:** Prooxidant-induced activation of TLR4 through c-Src/NFB coupling provides a basis for a molecular understanding of the initiation and maintenance of "sterile inflammation".

Disclosures: O.J. Igwe: None. **Y. Zhang:** None. **R. Karki:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.20/X10

Topic: E.05. Stress and the Brain

Title: Psychological stress increases indices of neuroinflammation

Authors: M. WARWICK, K. KORNACKER, L. THAN, *D. Y. LO;
Coe Col., Cedar Rapids, IA

Abstract: Ample evidence has shown that intense psychological stressors can increase inflammation within the brain, and that stress-induced neuroinflammation contributes to the development of a host of central nervous system disorders. Despite the established relationship between stress and neuroinflammation, the mechanisms underlying the pathogenic effects of stress-induced neuroinflammation remain unclear. To elucidate the effects of stress on neuroinflammation, immunocytochemistry experiments were performed to characterize the expression of various inflammation-related proteins in the brains of acutely- and chronically-stressed animals. Experiments using glial fibrilary acidic protein (GFAP) to assess astrocyte reactivity revealed increased GFAP immunoreactivity in the brains of both acutely- and

chronically-stressed animals in several brain regions, including the striatum and corpus callosum, and surrounding blood vessels throughout the brain. While we observed a noticeable difference in GFAP immunoreactivity between control and stressed groups, we did not notice a difference between acutely-stressed and chronically-stressed animals. Experiments looking at the expression of inflammatory markers NF-kappaB (NF-kB) and cyclooxygenase-2 (cox-2) showed that stress increases NF-kappaB expression throughout the brain in cells consistent with glia, neurons, and perivascular cells, and it increases cox-2 expression in neurons throughout the cortex. Interestingly, we observed an increase in cox-2 expression in the brains of chronically-stressed compared to acutely-stressed animals, suggesting that repeated stressors act to exacerbate brain inflammation. Taken together, our results show that stress has widespread inflammatory effects on the brain by modulating glial activity and upregulating the expression of several pro-inflammatory genes.

Disclosures: **M. Warwick:** None. **K. Kornacker:** None. **L. Than:** None. **D.Y. Lo:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.21/X11

Topic: E.05. Stress and the Brain

Support: NIMH 038752

NIMH 095380

NIMH 090236

Title: A pre-conditioning stress accelerates increases in mouse plasma inflammatory cytokines induced by stress

Authors: ***Y. CHENG**, R. JOPE, E. BEUREL;
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Abstract: Major depressive disorder is a prevalent disease that is inadequately treated with currently available interventions. Stress increases susceptibility to depression in patients and rodent models, which are also associated with aberrant activation of inflammation, specifically with increases in circulating IL-1β, IL-6, and TNFα. The two major goals were to measure changes in a broad panel of 19 cytokines, and to test if a pre-conditioning stress altered the inflammatory response to a subsequent stress. Administration of inescapable foot shocks

increased plasma levels of IL-1β, IL-6, TNF, IL-3, IL-10, IL-13, IL-17A, IL-5, GM-CSF, IL-12(p70), IFN-γ, MIP-1α, MIP-1β, IL-1α, IL-2, KC, RANTES and G-CSF, with peak levels occurring in the range of 6 to 12 hr after stress. Pre-conditioning the mice 24 hr prior with an equivalent stress resulted in similar magnitudes of increases in most cytokines as occurred after a single stress, but accelerated the increase, causing the levels of most cytokines to peak 1 hr after stress. These results demonstrate that a single stress induces the expression of many cytokines, and that sequential daily stresses accelerates the rate of cytokine production. Thus, stress broadly activates inflammation and the inflammatory response is more rapid following repeated stress, actions that may contribute to deleterious effects of stress on depression and other stress-linked diseases.

Disclosures: **Y. Cheng:** None. **R. Jope:** None. **E. Beurel:** None.

Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: Grants-in-Aid for Scientific Research (23390189)

Title: Repeated, not single, stress induces persistent polyi:c-induced allodynia and depressive-like behavior in rats

Authors: ***T. OKA**, T. CHIJIWA;
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Abstract: Background: When animals suffer from viral infections, they develop sickness response. Recent studies suggest that psychological stress can modulate the sickness response. However, it remains uncertain whether acute and chronic psychosocial stress have the same effect on viral infection-induced sickness responses. Methods: To address this question, we compared changes in polyI:C-induced sickness responses, such as fever, mechanical allodynia, and depressive-like behavior in rats that had been pre-exposed to single and repeated social defeat stress. Results: PolyI:C-induced fever was attenuated by the pretreatment with either single or repeated social defeat stress. In contrast, only the repeated stress group showed late-onset and prolonged mechanical allodynia lasting until 35 days after injection in the von Frey test and prolonged immobility time in the forced swim test 9 days post-injection. To assess the involvement of glucocorticoids and microglia in the delayed and persistent development of these

sickness responses in rats exposed to repeated stress, we also investigated the effect of pretreatment with RU486, a glucocorticoid receptor antagonist, and minocycline, an inhibitor of microglial activation, on polyI:C-induced allodynia and depressive-like behavior. Pretreatment with either drug inhibited both the delayed allodynia and depressive-like behavior. Conclusions: The present study demonstrates that repeated, but not single, social defeat stress followed by systemic polyI:C administration induced prolonged allodynia and depressive-like behavior in rats. Stress-induced corticosterone and microglial activation may play a pivotal role in this phenomenon.

Disclosures: **T. Oka:** None. **T. Chijiwa:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

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Program#/Poster#: 714.23/X13

Topic: E.05. Stress and the Brain

Support: NIH Grant MH099580

Title: Repeated stressor exposure differentially effects microglia and peripheral macrophages proinflammatory cytokine production and the regulation by norepinephrine

Authors: D. F. BARNARD, *J. D. JOHNSON;
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Abstract: The elevation of proinflammatory cytokines have been implicated in a wide variety of physiological and behavioral diseases, such as depression and PTSD. Norepinephrine plays a critical role in the regulation of proinflammatory cytokines with studies demonstrating it has both stimulatory and inhibitory effects depending on the activation state of the immune cell. In fact, norepinephrine release during times of stress mediates the elevation of both central and peripheral cytokine production, which is critical for the onset of depression-like and anxiety-like symptoms in multiple animal models. Adrenergic receptor expression and signaling changes after stressor exposure, yet it remains unknown how the regulation of proinflammatory cytokines by norepinephrine might be altered following repeated stressor exposure. Studies here examine how stress influences norepinephrine's ability to regulate basal and LPS-induced cytokine production from both peripheral and central immune cells. Rats were exposed to a four-day stress protocol and sacrificed 24h following the last stressor. Microglia from the hippocampus as well as peritoneal phagocytes were isolated and plated. Cells were treated for 20h with media or

LPS with or without three doses of norepinephrine. Stressor exposure significantly suppressed LPS-induced IL-1 production in microglia while having no effect on TNF-alpha production. Interestingly, the highest dose of norepinephrine enhanced LPS-induced IL-1 production in microglia from both control and stressed animals but had no effect on TNF-alpha production. Alternatively, the effects of stress and norepinephrine's ability to regulate cytokine production in peripheral immune cells drastically differed from microglia. Stress enhanced LPS-induced IL-1 production from peritoneal cells and norepinephrine enhanced this production more pronouncedly in stressed animals compared to controls. Additionally, stress significantly enhanced LPS-induced TNF-alpha production from peritoneal phagocytes and this was inhibited by the high dose of norepinephrine in both control and stress groups. These results indicate that the ability of norepinephrine to regulate LPS-induced cytokine production is specific to both cell type (microglia or peripheral macrophage) as well as the cytokine type.

Disclosures: D.F. Barnard: None. **J.D. Johnson:** None.

Poster

714. Somatic Correlates of Stress

Location: Hall A

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Topic: E.05. Stress and the Brain

Support: T32 MH 019929

T32-084907

American Federation for Aging Research (AFAR)

Swiss National Science Foundation (SNF)

Title: Basal HPA axis activity is related with responses of inflammatory gene expression to acute stress

Authors: X. CHEN[1], C. M. MCINNIS[1], D. WANG[2], L. HANLIN[1], D. GIANFERANTE[1], M. V. THOMA[1], *N. ROHLEDER[1];
[1]Psychology, Brandeis Univ., Waltham, MA; [2]Davis Sch. of Gerontology, USC, Los Angeles, CA

Abstract: Introduction: Diurnal hypothalamus-pituitary-adrenal (HPA) axis activity, which is characterized by an increase during the first hour after awaking (cortisol awakening response;

CAR), and a gradual decline afterwards, has been found to be altered in individuals with chronic stress or psychiatric disease, and to be predictive of health outcomes. The mechanism linking basal activity with health remains unclear. In the present study, we investigated whether basal HPA axis activity was related with response and habituation of inflammatory gene expression to acute stress in humans. **Methods:** We recruited n=36 healthy individuals from two age groups (Young adult group: 13 male/11 female, mean age 20.75 yrs. ± 3.66 SD, BMI 24.14 kg/m^2 ± 3.25 SD; Older adult group: 5 male/7 female, mean age 50.17 yrs. ± 9.46 SD; BMI 25.52 kg/m^2 ± 2.86 SD) and collected salivary cortisol at 6 points in two successive days: immediately and 0.5h, 1h, 4h, 9h and 13h post-awakening. Participants were further exposed to the laboratory stressor Trier Social Stress Test (TSST) on two consecutive days. On both stress days peripheral blood was taken before, 30 and 120 min post TSST, and gene expression of interleukin (IL)-6, IL-1β, nuclear factor (NF)-κB, and IκB was measured using qPCR. **Results:** As reported before, expression of all genes increased after TSST1 and habituated after TSST2. A steeper CAR was related with higher IL-6 expression on both days (F= 6.00, p=0.021). Steeper diurnal cortisol decline was a marginally significant predictor of higher IL-6 at 120 min post-TSST1 (β=0.36, p=0.06), and of higher NF-κB expression post stress on both days (F= 3.22, p=0.08). Higher total daily cortisol output was a significant predictor of lower IL-1β response at 30 min (β=-0.60, p=0.003) and 120 min (β=-0.38, p=0.07) post-TSST2, and stronger IL-1β habituation at 30 min post-TSST (β=0.49, p=0.015), and it also marginally predicted lower IκB at 30mins post-TSST2 (β=-0.35, p=0.09), all controlling for age and gender. **Conclusion:** Results show that basal HPA axis activity was related with inflammatory gene expression in response to acute stress. Considering the importance of peripheral inflammation for health, this might be an important step in bridging the gap in the current literature regarding health effects of acute stress reactivity and health outcomes of basal HPA axis activity. Future work will employ longitudinal studies to understand the direction of these relationships.

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Poster

714. Somatic Correlates of Stress

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 714.25/X15

Topic: E.05. Stress and the Brain

Support: DARPA Grant W911NF1010093

Title: Sphingosine-1-phosphate receptors are novel regulators of the hypothalamic-pituitary-adrenal (HPA) axis

Authors: *N. SOTUYO[1,2], S. LUZ[2], S. BHATNAGAR[2,1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Div. of Stress Neurobio., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: In the past decade, sphingolipid receptors (sphingosine-1-phosphate receptors 1-5; S1PR1-5) have become popular therapeutic targets for multiple sclerosis and other diseases due to their important roles in the cardiovascular and immune systems. However, the role of sphingolipid signaling in the central nervous system remains poorly understood. Fingolimod (FTY720) is an FDA-approved synthetic sphingolipid analog used to treat multiple sclerosis (MS) by acting as a functional S1PR1 antagonist on autoreactive T lymphocytes, leading to their sequestration in lymph nodes. Interestingly, patients treated with fingolimod also report less depression and anxiety than with other MS treatments (Montelban et al., 2011), which may be indicative of additional effects in the CNS. We were interested in whether fingolimod has any effects on the hypothalamic-pituitary-adrenal (HPA) axis, which has also been shown to be dysregulated in a subset of MS patients with major depressive disorder (e.g. increased cortisol levels, and abnormal cortisol awakening responses and dexamethasone suppression tests). In the current studies, we sought to characterize the effect of fingolimod on the HPA axis basally and in the context of various stressors. Specifically, we measured plasma levels of ACTH and corticosterone before, during, and after novel restraint stress and the social interaction test following either acute (single) or repeated intraperitoneal fingolimod administration. Interestingly, an acute injection of 2.5 mg/kg fingolimod increased basal HPA hormones, but no additional effect of restraint was observed. However, with repeated injections (3 days of 2.5mg/kg/day ip) restraint-induced ACTH was significantly increased. Furthermore, fingolimod increased the number of activated cells in the paraventricular nucleus of the hypothalamus (PVN) consistent with its role in regulating ACTH. Increased neuronal activity was also observed in the medial prefrontal cortex and basolateral amygdala, regions important in regulating the stress response. In sum, these results demonstrating fingolimod's time-dependent effects on the HPA axis may highlight a novel role for sphingolipid signaling as a central regulator of the HPA axis. Further elucidation of the mechanisms underlying this HPA activation by S1P receptors will be important for developing effective therapies for stress related psychiatric diseases, including but not limited to major depressive order seen in HPA-dysregulated MS patients.

Disclosures: **N. Sotuyo:** None. **S. Luz:** None. **S. Bhatnagar:** None.

Poster

714. Somatic Correlates of Stress

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Topic: E.05. Stress and the Brain

Support: NIA grant R01-AG038043

Title: Stress response profiles in women during the active hormone and inactive no-hormone weeks of hormonal contraception

Authors: *A. E. YCAZA, S. E. NIELSEN, M. MATHER;
Davis Sch. of Gerontology, USC, Los Angeles, CA

Abstract: Cortisol responses to stress vary across the menstrual cycle and with hormonal contraception (HC) use. For example, women on HC and in the low hormone phase of the menstrual cycle experience lower cortisol responses to stress compared with women during the high hormone phase of the menstrual cycle. This pattern suggests that the low ovarian output experienced during HC use may be related to the reduced cortisol response to stress. However, it is unknown whether this reduced cortisol response to stress persists when HC-induced ovarian suppression is removed during the no-hormone inactive days of HC. To examine the effect of HC phase (active vs. inactive) on the adrenal and sympathetic stress response, we tested 41 women during either the active (hormone days; n = 23) or inactive (n = 18) phases of their HC regimens. Women had been using HC (monophasic oral pill or vaginal ring) for at least 2 months. Stress was induced via the cold pressor task (holding one's hand in ice water) for up to 3 minutes. Saliva samples were collected immediately before stress onset (baseline), immediately after stress exposure (post-stress 1), and 15 minutes after stress onset (post-stress 2). Salivary alpha-amylase (sAA) and cortisol was measured in all 41 participants, and progesterone (P4) was measured in 40 participants (active: n = 22; inactive: n = 18). A series of chi-square analyses revealed a similar number of women in each HC phase experienced increases in cortisol and sAA in response to cold pressor stress. Conversely, more women experienced increases in P4 during the active phase of HC than during the inactive phase. Next, a series of 2 (HC phase) x 3 (salivary sample time point) mixed ANOVAs revealed a main effect of stress on cortisol, but not on P4 or sAA. There also was no main effect of HC phase on cortisol, P4, sAA, nor any interactions. To better characterize response differences, we then compared the change score in cortisol and P4 responses between HC phases. Independent t-tests revealed no difference in magnitude of cortisol change between HC phases. However, P4 response from baseline to post-stress 1 was significantly different between HC phases, with active women experiencing an increase and inactive women experiencing a decrease. Despite evidence that women not using HC experience greater stress responses than women using HC, the comparable cortisol and sAA response to stress in both HC phases suggests that the blunting effect of HC on the stress

response persists into the inactive, no hormone phase of a HC regimen. Surprisingly, however, the P4 response to stress is larger during the active phase of HC, which may relate to the anxiolytic properties of P4 and the ability of HC to stabilize mood.

Disclosures: **A.E. Ycaza:** None. **S.E. Nielsen:** None. **M. Mather:** None.

Poster

715. Estrogen Signaling and Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 715.01/X17

Topic: E.03. Behavioral Neuroendocrinology

Support: KAKEN #23240057 to SO

KAKEN #15H01844 to SO

Title: Pubertal activation of estrogen receptor α in the medial amygdala is necessary for the expression of male-type social behavior in adult mice

Authors: K. SANO[1], M. NAKATA[1], S. MUSATOV[2], S. TSUKAHARA[3], N. YAMAGUCHI[4], *T. SAKAMOTO[5], S. OGAWA[1];
[1]Lab. of Behavioral Neuroendocrinology, Univ. of Tsukuba, Tsukuba, Japan; [2]Mol. Neurosurg., Weill Cornell Univ. Med. Col., New York, NY; [3]Grad. Sch. of Sci. and Engin., Saitama Univ., Saitama, Japan; [4]Dept. of Pharmacol., Aich Med. Univ., Nagakute, Japan; [5]Kyoto Tachibana Univ., Kyoto, Japan

Abstract: Testosterone is known to play a central role in the facilitation of male-type social behavior, such as sexual and aggressive, and the development of their neural bases in male mice. Moreover, the action of testosterone via estrogen receptor (ER) α, after being aromatized to estradiol in the brain, is suggested to be crucial for the full expression of these behaviors. We previously reported that knocking down of ERα in adulthood with the use of virally mediated RNAi method in the medial amygdala (MeA) had no effect on either behavior (Sano et al. EJN, 2013). Recent studies have shown that testosterone stimulation in pubertal period may play a critical role for full expression of male social behavior in adulthood. However, it is still not known whether and in which brain region(s) ERα is involved in this developmental effect of testosterone. In the present study, we examined the effects of pre-pubertal knockdown of ERα in the MeA. At the age of 21 days, gonadally intact male mice (ICR/Jcl) were bilaterally injected either with adeno-associated viral vector silencing ERα or a control vector in the MeA. All mice

were then tested for their sexual and aggressive behaviors starting at 12 weeks old. We found that pre-pubertal knockdown of ERα in the MeA reduced both sexual and aggressive behaviors. Furthermore, the number of MeA neurons examined in adult was reduced to the level similar to that of female. These results collectively suggest that although it may not be required at the time of testing, ERα activation in the MeA by aromatized testosterone during pubertal period is necessary for the full masculinization of this region that is essential for the expression of male-type social behavior later in adulthood. In addition, we also examined the pre-pubertal knockdown effects of ERβ in the MeA on the male social behavior. Unlike that of ERα, knocking down of ERβ during pre-pubertal period did not affect the expression of either sexual or aggressive behavior in adulthood.

Disclosures: **K. Sano:** None. **M. Nakata:** None. **S. Musatov:** None. **S. Tsukahara:** None. **N. Yamaguchi:** None. **T. Sakamoto:** None. **S. Ogawa:** None.

Poster

715. Estrogen Signaling and Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: KAKEN #23240057 to SO

KAKEN #15H01844 to SO

Title: Role of estrogen receptor β in the dorsal raphe nucleus in the regulation of female sexual behavior in mice

Authors: C. MORIMOTO[1], K. SANO[1], M. NAKATA[1], S. MUSATOV[2], N. YAMAGUCHI[3], T. SAKAMOTO[4], *S. OGAWA[1];
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Abstract: Estrogen regulates the expression of female sexual behavior by acting through estrogen receptors, ERα and ERβ. Both receptors are widely but somewhat differentially distributed in various brain regions. It is well known that ERα in the hypothalamic ventromedial nucleus plays a crucial role for the facilitation of female sexual behavior during the estrus phase. On the other hand, neuroendocrine mechanism to suppress sexual receptivity in non-estrous

females, particularly on the day after the behavioral estrus, still remains unclear. Our previous studies have shown that global ERβ knockout (βERKO) female mice maintained high levels of lordosis behavior on the day after behavioral estrus (Ogawa et al., PNAS, 1999). Therefore, it is hypothesized that ERβ may be involved in the inhibition of lordosis, but responsible brain regions have not been identified yet. In this study, we site-specifically knocked down the ERβ expression (βERKD) in the dorsal raphe nucleus (DRN) with adeno-associated viral (AAV) vector expressing a small hairpin RNA targeting ERβ. We examined the expression of sexual behavior in ovariectomized mice with steroid-priming which mimicked the hormonal conditions of the day of behavioral estrus (Day 1) and the day after behavioral estrus (Day 2). βERKD mice showed similarly high levels of lordosis quotient (LQ, number of lordosis / number of mounts and intromissions) and receptivity score (the average of 15 lordosis scores (rated as 0-3) on Day 1 as mice injected with control vectors. However, βERKD mice continuously showed high levels of LQ and receptivity score on Day 2, when control mice showed a great reduction in both measurements compared to Day 1. These results suggest that the expression of ERβ in the DRN may be involved in the inhibitory regulation of female sexual behavior on the day after behavioral estrus.

Disclosures: **C. Morimoto:** None. **K. Sano:** None. **M. Nakata:** None. **S. Musatov:** None. **N. Yamaguchi:** None. **T. Sakamoto:** None. **S. Ogawa:** None.

Poster

715. Estrogen Signaling and Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: KAKEN #2324057 to SO

KAKEN #15H01844 to SO

Title: Effect of site-specific knockdown of estrogen receptor α or β in the medial preoptic area on postpartum aggression in female mice

Authors: *K. NAGATA[1], Y. MIYATA[1], K. SANO[1], S. MUSATOV[2], S. OGAWA[1]; [1]Lab. of Behavioral Neuroendocrinology, Univ. of Tsukuba, Tsukuba-Shi, Japan; [2]Mol. Neurosurg., Weill Cornell Med. Col., New York, NY

Abstract: Estrogen plays a critical role not only in the induction of female sexual behavior but also in the regulation of various other social behaviors including maternal behavior. It is well known that postpartum lactating female mice show fierce aggressive behavior towards intruder male mice as part of their maternal caring behavior. However, neuroendocrine mechanism of postpartum aggression is not completely understood. In the present study, we aimed to determine whether two types of estrogen receptors (ER), ERα and ERβ, both highly expressed in the medial preoptic area (MPOA) that is known to play a critical role for maternal behavior, may also be involved in the regulation of postpartum aggression. We site-specifically knocked down ERα or ERβ in the MPOA by bilaterally injecting adeno-associated viral (AAV) vector expressing a small hairpin RNA targeting either ERα or ERβ in ICR/Jcl virgin female mice. All mice were then mated and individually housed once pregnancy was confirmed. On the day of parturition, defined as postpartum day (PPD) 0, litters were culled to as close to 10 pups as possible. Dams were tested against an olfactory bulbectomized male intruder mouse in a resident-intruder paradigm on PPD 1, 3, 5, 7, 9, 13, and 17. Control dams showed high levels of aggression during the early period of lactation (PPD 3, 5, and 7), and their levels of aggression declined thereafter. Dams of the ERα knockdown group exhibited similar levels of aggression as control mice throughout the postpartum period. In contrast, knockdown of ERβ greatly increased the levels of aggression compared to those of the control group. These results suggest that ERβ expressed in the MPOA may be involved in inhibitory modulation of the levels of aggression in postpartum female mice.

Disclosures: **K. Nagata:** None. **Y. Miyata:** None. **K. Sano:** None. **S. Musatov:** None. **S. Ogawa:** None.

Poster

715. Estrogen Signaling and Behavior

Location: Hall A

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Topic: E.03. Behavioral Neuroendocrinology

Support: KAKEN #23240057 to SO

KAKEN #15H01844 to SO

Title: The role of estrogen receptor β in adult medial amygdala in the regulation of male social behavior

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Abstract: It is well known that testosterone plays an essential role in the regulation of male-type social behavior. Moreover, estrogen receptors (ERs) are known to mediate behavioral effect of testosterone, after aromatization of testosterone to estradiol in the brain. Previous studies using knockout mouse models suggested that two types of ERs, ERα and ERβ, play different roles in the regulation of male-type social behavior including sexual preference to receptive female, and sexual and aggressive behaviors. To identify brain site(s) responsible for ERα mediated action of testosterone, we previously conducted site-specific knockdown of ERα using virally mediated RNAi technique (Sano et al. EJN, 2013). However, it is still not known about brain site-specific role of ERβ in the regulation of male social behavior. In the present study, we examined the effects of site-specific knockdown ERβ in the medial amygdala (MeA), in which ERβ is abundantly expressed, and known to be responsible for processing of social cues. Gonadally intact adult male mice (ICR/Jcl) were bilaterally injected either with adeno-associated viral vector silencing ERβ (βERKD) or a control vector in the MeA. Three weeks after injection, animals were tested for preference between a receptive female (ovariectomized and primed with estradiol and progesterone) and a gonadally intact male (PTFM), and between a receptive female and a non-receptive (ovariectomized) female (PTFF). After preference tests, we conducted sexual and aggressive behavior tests. We found that ERβ knockdown in the MeA disrupt male-typical sexual preference. Unlike in control mice which showed preference to a receptive female over a non-receptive female, βERKD mice sniffed both females at a comparable level in the PTFF test. On the other hand, knockdown of ERβ did not affect preference during PTFM test and expression of sexual and aggressive behaviors. To further investigate functional significance of our finding, we additionally tested whether male mice preferentially mate with a receptive female when receptive and non-receptive females were simultaneously introduced to their home cage. As observed in the PTFF test, βERKD mice showed no preference to a receptive female in terms of mount and intromission frequency whereas control mice tended to show more sexual behavior toward a receptive female than to a non-receptive female. These results suggest that ERβ activation in the MeA at the time of testing in adult is essential for the expression of male-type sexual preference.

Disclosures: **M. Nakata:** None. **K. Sano:** None. **S. Musatov:** None. **N. Yamaguchi:** None. **T. Sakamoto:** None. **S. Ogawa:** None.

Poster

715. Estrogen Signaling and Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: KAKEN #23240057 to SO

KAKEN #15H01844 to SO

Title: Site-specific action of testosterone via estrogen receptor β, not α, in the medial preoptic area is required for the full expression of aggressive behavior in male mice

Authors: *N. YAMAGUCHI[1], K. SANO[2], M. NAKATA[2], S. MUSATOV[3], T. SAKAMOTO[4], S. OGAWA[2];
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Abstract: The expression of male-type social behavior such as sexual and aggressive, are highly dependent on the action of testosterone. Testosterone is known to activate not only androgen receptors but also estrogen receptors (ER), ERα and ERβ, after being aromatized to estradiol in the brain. A series of our recent studies revealed that two types of ERs in different brain regions might be involved to regulate each of sexual and aggressive behaviors differently. Among them, we have reported that activation of ERα in the medial preoptic area (MPOA) at the time of testing in adult is necessary for the facilitation of male sexual, but not aggressive, behavior (Sano et al. EJN, 2013). However, mechanisms of testosterone action in the MPOA for the expression of male aggressive behavior still remain unclear. Since we have found that activation of ERα during pubertal period in the medial amygdala may be crucial for full expression of sexual and aggressive behaviors (Sano et al., SfN abstract, 2015), it is possible that pre-pubertal knockdown of ERα in the MPOA may also affect aggressive behavior in adulthood. Furthermore, ERβ highly expressed in the MPOA might be involved in the regulation of aggressive behavior by aromatized testosterone. Thus in this study, we site-specifically knocked down ERα or β in the MPOA during pre-pubertal period and examined the effect on the expression of male aggressive behavior. At the age of 21 days, gonadally intact male mice (ICR/Jcl) were bilaterally injected either with adeno-associated viral vectors silencing ERα or ERβ, or a control vector in the MPOA. Starting at the age of 12 weeks, all mice were tested for their aggressive behavior. Surprisingly, knockdown of ERβ reduced the levels of aggression while the knockdown of ERα had no effect on the expression of aggressive behavior. These results indicate that testosterone action via ERβ, but not ERα, in the MPOA is required for the full expression of male aggressive behavior. Taken together with our previous studies, it is suggested that ERα and ERβ in the

MPOA may be responsible for the differential regulation of male sexual and aggressive behaviors by testosterone.

Disclosures: **N. Yamaguchi:** None. **K. Sano:** None. **M. Nakata:** None. **S. Musatov:** None. **T. Sakamoto:** None. **S. Ogawa:** None.

Poster

715. Estrogen Signaling and Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS 0849102

Title: Estrogen receptor GPR30/GPER colocalizes with isotocin in the preoptic area of a social fish

Authors: *L. A. MANGIAMELE[1], R. R. THOMPSON[2];
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Abstract: Estradiol can rapidly (within 1 hr) modulate social and reproductive behaviors in both mammalian and non-mammalian species, potentially through actions on neuroendocrine cell populations in the hypothalamus and preoptic area. For example, recent studies have shown that estradiol can stimulate central and peripheral release of the neuropeptides oxytocin (the mammalian homologue of isotocin, IT) and vasopressin (the mammalian homologue of vasotocin, VT), which are potent modulators of social behaviors in all species thus far examined. The mechanism by which these rapid neuroendocrine effects occur is, however, unclear. One possibility is that estradiol stimulates rapid neuropeptide release via a non-genomic mechanism through receptors such as GPR30 (also known as GPER), a G protein-coupled membrane estrogen receptor. To address this issue, we first localized GPR30 in the brain of male goldfish via immunohistochemistry. We found that GPR30 is robustly expressed throughout the preoptic area (POA), a highly conserved node in the social brain network. Using an anti-oxytocin antibody to label neurons that produce isotocin, we then asked whether GPR30 was expressed specifically in isotocin-producing cell populations. We found that virtually all GPR30-positive cells and fibers in the POA were also IT-positive. Some POA neurons had double-labeled fibers with varicosities, suggesting that GPR30 may be co-localized with the neuropeptide's axonal secretory vesicles. Preadsorption of the anti-oxytocin antibody with excess VT did not block

GPR30 or IT immunoreactivity or co-immunolabeling, however, preadsorption of the anti-oxytocin antibody with excess IT eliminated IT immunoreactivity but not GPR30 immunoreactivity. We observed similar patterns of GPR30/isotocin co-localization in males sacrificed both during and outside of the breeding season. Together, these observations suggest that GPR30 has the potential to mediate the rapid neuroendocrine effects of estradiol in the preoptic area, including the rapid release of isotocin, to influence vertebrate sociality.

Disclosures: L.A. Mangiamele: None. **R.R. Thompson:** None.

Poster

715. Estrogen Signaling and Behavior

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Program#/Poster#: 715.07/X23

Topic: E.03. Behavioral Neuroendocrinology

Title: Recurrent clusters of gene expression characterize the impact of the estrous cycle in the rat brain

Authors: A. KACPURA[1], R. SCHMIDT[2], L. WELCH[2], *S. DE LACALLE[1];
[1]Biomed. Sci., Heritage Col. of Osteo. Med., Athens, OH; [2]Electrical Engin., Russ Col. of Engin., Athens, OH

Abstract: The roles of hormonal and genetic factors in the emergence of sex differences in susceptibility to mental disorders, mood and cognitive disturbances are far from understood. While it is not known why major depressive disorder affects twice as many women as men over the course of a lifetime, fluctuating reproductive hormones are a potential factor (Joffe & Cohen, 1998), perhaps through changes in neurotransmitters involved in mood regulation (Freeman et al., 2006; Daly et al., 2003). We set out to identify patterns of gene expression across the estrous cycle, with the goal of obtaining a point of reference to understand the connection between hormonal cyclicity and mental health in aging. We processed the basal forebrain, dorsal hippocampus and frontal cortex regions of female rats at 10 am and 6 pm on proestrus, and at 10 am on estrus (n=4 in each group), using Affimetrix Rat Genome 230 2.0 Array. A comparison of the three time points across all brain regions revealed 198 probes and 106 genes differentially expressed with a nominal p-value under the 0.05 threshold. Applying the Bayesian estimation of temporal regulation (BETR) algorithm we identified 465 probes and 273 genes differentially expressed, with probability greater than 0.99, across the three time points in all brain regions. Compared to this overall brain expression, each brain region exhibited larger number of differentially expressed probes and genes: 693 probes and 422 genes in the frontal cortex; 798

probes and 529 genes in the basal forebrain, and 1642 probes and 1054 genes in the hippocampus. The hippocampus contained the most differentially expressed probes and genes when using a BETR probability threshold of 0.99. Fuzzy clustering of differentially expressed probes identified by the BETR algorithm was conducted using the Bioconductor package Mfuzz 2.18.0, resulting in the identification of nine expression patterns for each of the specific areas of the brain and across the brain as a whole. Clearly, the impact of the estrous cycle on gene expression in the central nervous system appears to follow a tightly regulated pattern with unique features in the different brain regions analyzed, and it also seems to be more extensive than anticipated.

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Poster

715. Estrogen Signaling and Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Title: Changes in socio-sexual interactions during transition from non-estrus to estrus in devocalized as well as vocalizing hormone-treated, ovariectomized rats housed in a semi-natural environment

Authors: ***O. LE MOENE**[1], E. SNOEREN[2], X. CHU[2], A. AGMO[2];
[1]Institutt for Psykologi, Universitetet I Tromsø, Tromso, Norway; [2]Fac. of Hlth. and Sci., Tromsø, Norway

Abstract: Previous studies in intact females revealed a sudden change in behavior immediately preceding the first lordosis displayed during estrus. The purpose of the present study was to determine whether this sudden change occurs also in ovariectomized females after sequential estrogen - progesterone treatment. Five groups of 7 rats (3 males and 4 females) were housed in a semi-natural environment for 8 days. Some of the individuals were devocalized prior to experiment. Females were injected with 18 µg/kg of estradiol benzoate 48 h prior to observation and with 1 mg/rat of progesterone 4 h before observations. Socio-sexual behaviors during the 8 minutes preceding the first lordosis of each female and the 8 minutes following it were recorded. Females demonstrated more paracopulatory behaviors during the estrus than before it, but also more nose-off and more flight. In parallel, males performed more pursuits, more mounts and more nose-off during estrus. Further analysis revealed that most of the behavioral changes happened between the last minute of the pre-estrus period and the first minute of the estrus; and

for some of them even between the last 30 sec of the pre-estrus and the first 30 sec of the estrus. For the first 8 minutes of the estrus period, the lordosis quotient was 97±16%. This demonstrates that once a female had displayed the first lordosis, she responded with lordosis to almost all mounts. Thus, the transition from non-estrus to estrus occurred in less than one minute, and females achieved instantaneously maximum receptivity. During the estrus, the number of agonistic behaviors was correlated to the number of sexual behaviors performed by the females. One explanation may be that the females in estrus attracted more males, then by rejecting unpreferred males, they increased their agonistic behaviors. This relationship was not observed among the male behaviors, which suggests that the rise in female agonistic behaviors is indeed due to male sexual solicitation. No effect of devocalization was found on socio-sexual behaviors during the transition period. Moreover, males did not show any preference for vocalizing females compared to devocalized ones. Conversely to what has been observed in most studies conducted in small cages and with forced sexual interactions, the behavioral transition from non-estrus to estrus is instantaneous rather than gradual in a semi-natural environment in which female herself determines whether to copulate or not. Because this pattern exists both in intact females and in females with induced estrus, we can conclude that the behavioral changes observed are not directly related to momentaneous changes in hormones levels.

Disclosures: **O. Le Moene:** None. **E. Snoeren:** None. **X. Chu:** None. **A. Agmo:** None.

Poster

715. Estrogen Signaling and Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: CNPq

CAPES

FAPERGS

Title: Differences in oxytocin, vasopressin, dopamine and estrogen receptor expression in female and male oxytocin knockout (OTKO) mice

Authors: ***A. B. LUCION**[1], V. LAZZARI[3], J. ZIMMERMANN-PERUZATTO[2], R. O. BECKER[3], S. ALMEIDA[4], M. GIOVENARDI[3];

[2]Physiol., [1]Univ. Federal do Rio Grande Sul UFRGS, Porto Alegre, Brazil; [3]Physiol., [4]Mol. Biol., Univ. Federal de Ciencias da Saude de Porto Alegre, Porto Alegre, Brazil

Abstract: Previous studies showed reduced sexual behavior in oxytocin knockout (OTKO) female mice, but not males. Present study aimed to analyze the expression of oxytocin (OXTR), vasopressin 1A (AVPR1a), dopamine D2 (D2R), alpha estrogen (ERα), and beta estrogen (ERβ) receptors in the prefrontal cortex (PFC), hippocampus (HPC), hypothalamus (HPT), and olfactory bulb (OB) of C57BL/6 mice. The animals (n=7/group) were genotyped (WT and OTKO groups); decapitated; and brain areas were collected. Total RNA was extracted and cDNA was produced. Amplification was performed by real-time quantitative polymerase chain reaction. Data are presented as mean ± SE; Mann-Whitney test was used for statistical analysis. Male mice results: OTKO group had increased OXTR expression in the HPC (WT: 9.96±6.35; OTKO: 69.84±40.09); in the other areas OXTR expression was not different between groups. D2R decreased expression in HPC (WT: 6.26±3.95; OTKO: 0.28±0.12); no differences were detected in other areas. OTKO group had increased ERβ expression in PFC (WT: 1.23±0.30; OTKO: 4.73±1.05); no differences were detected in the other areas. AVPR1a and ERα showed no differences between groups in all areas. Female mice results: OTKO group had decreased OXTR expression in HPC (WT: 1.56±0.51; OTKO: 0.52±0.08), while other areas had no differences. OTKO group had increased expression of AVPR1a in HPC (WT: 1.36±0.36; OTKO: 3.97±0.77) and decreased in HPT (WT: 1.35±0.36; OTKO: 0.26±0.09), no differences in other areas. In the PFC, OTKO group had decreased ERα (WT: 1.24±0.30; OTKO: 0.43±0.21) and ERβ expression (WT: 1.40±0.39; OTKO: 0.40±0.132); no differences in other areas. No differences in the expression of D2R were shown. The lack of oxytocin in the OTKO mice affects differently male and females: while OXTR is increased in males HPC, it is decreased in females HPC; while ERβ is increased in males PFC, it is decreased in females PFC. These opposite results can be an interesting way of behavioral modulation of sexual behavior. The dopamine receptors in the HPC were affected only in OTKO males. Differences in AVPR1a and ERα expression were detected only in females. The lack of oxytocin induces sex specific changes in receptors in specific brain areas that could be related to the behavioral changes observed in OTKO animals.

Disclosures: **A.B. Lucion:** None. **V. Lazzari:** None. **J. Zimmermann-Peruzatto:** None. **R.O. Becker:** None. **S. Almeida:** None. **M. Giovenardi:** None.

Poster

715. Estrogen Signaling and Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Title: A comparison of maternal hormonal influences in two animal models of autism

Authors: *H. GARMAN[1,2], A. LEE[5,2], J. KASS[5,3], P. WHITAKER-AZMITIA[5,4]; [2]Integrative Neurosci. in Psychology, [1]Stony Brook Univ., Stony Brook, NY; [3]Integrative Neurosci. in Psychology, Stony Brook Univ., Stony Brook University, NY; [4]Integrative Neurosci. in Psychology, Psychiatry, Stony Brook Univ., Stony Brook, NY; [5]Stony Brook Unversity, Stony Brook, NY

Abstract: The maternal environment associated with the neuroendocrine system has been proposed to play a role in the etiology of Autism Spectrum Disorders (ASD). Characteristics that typify ASD are heterogeneous with an increased incidence in males and several etiological elements are likely to be involved. Progesterone and testosterone are sex hormones involved in brain development which have been implicated in ASD. Specifically, low maternal progesterone (as evidence by increased obstetrical complications) or high testosterone (as proposed in the extreme male hypothesis) have been proposed as possible causes of ASD. Here, we compared a model of low progesterone (lowP), using the progesterone antagonist, RU-486 (5 mg/kg) or high testosterone (T) using 17β-estradiol (5µg/ kg), which masculinizes rodents, in order to test the hypothesis that these could be contributing factors to ASD. Litters from six Sprague-Dawley rats were cross-fostered and divided into three equal groups of 20 pups each: and injected subcutaneously on postnatal days 1, 2 and 5 (equivalent to human third trimester). We observed their physical, social, and motor development using an array of behavioral testing relevant to ASD. Both lowP and T showed deficits in social bonding characteristic of animal models of ASD, including huddling and return to dam. Importantly, females in the lowP group showed less bonding than the males; this was not seen in the T group nor the control group. Both groups showed increased stereotypy on PND 27 compared to the control group. Results showed body weight differences across groups. The lowP group showed increased body weight, whereas the T group showed decreased body weight compared to the control group. Using negative geotaxis at PND's 7, 8 and 11 as a test for motor development, T showed accelerated motor development compared to control while lowP showed a delayed motor development compared to both groups. Our results show that hormones in the maternal environment may contribute to the social behavioral changes which are hallmark features of ASD. However, there are also differences which could explain the spectrum nature of ASD. Specifically, these behavioral results suggest that low progesterone may serve as a model for characterizing behaviors specific to females with ASD. On the other hand, high testosterone may model early increased brain development, seen in certain cases of ASD. These results suggest a disruption in the neuroendocrine system leading to changes in physical development, motor skills, and social behavior. Further investigation into these animal models may be a step forward in understanding the significance of maternal environment and risk of ASD.

Disclosures: **H. Garman:** None. **A. Lee:** None. **J. Kass:** None. **P. Whitaker-Azmitia:** None.

Poster

715. Estrogen Signaling and Behavior

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GMO7163

Title: Estradiol-G protein-coupled estrogen receptor 1 facilitation of sexual receptivity via direct regulation of the orphanin FQ/N-ORL-1 system in the arcuate nucleus of the hypothalamus

Authors: ***D. N. TRAN**, N. LONG, C. SEREY, K. SINCHAK;
California State Univ. Long Beach, Long Beach, CA

Abstract: In the ovariectomized (OVX) rat primed with 2 μg estradiol benzoate (EB), sexual receptivity (lordosis) can be facilitated within 30 minutes by infusing non-esterified 17β-estradiol (E2) into the arcuate nucleus of the hypothalamus (ARH). Infusion of the G protein-coupled estrogen receptor 1 (GPER; aka GPR30) agonist, G1, mimicked E2 facilitation of lordosis and was blocked by pretreatment with a GPER antagonist G15 (Long, et al., 2014 Horm Behav 66:663). EB-priming initially inhibits lordosis through rapidly activating ARH β-endorphin (β-END) neurons to activate μ-opioid receptors (MOP) in the medial preoptic nucleus (MPN). Forty-eight hours later, E2 infused into the ARH acts through GPER to inhibit ARH β-END neurons facilitating lordosis. A single high dose of EB (5-50 μg) also facilitates lordosis via orphanin FQ (OFQ/N; aka nociception) activating opioid receptor-like receptor 1 (ORL-1) that inhibits ARH β-END neurons. We therefore hypothesized that E2-GPER facilitation of lordosis is also mediated via OFQ/N activation of ORL-1 in the ARH. EB-primed (2 μg) OVX rats received sequential ARH infusions 47.25 (DMSO or UFP-101 (ORL-1 antagonist)) and 47.5 (DMSO, G1, or E2) hours later and then tested 30 minutes later for sexual receptivity. G1 and E2 ARH infusions facilitated lordosis and reduced MPN MOP activation. Pretreatment with UFP-101 prevented G1 and E2 MPN MOP deactivation and facilitation of lordosis, indicating the OFQ/N-ORL-1 system mediates E2-GPER facilitation of lordosis. The E2-GPER induced OFQ/N-ORL-1 inhibition of the β-END neurons is independent of progesterone receptor (PR) activation since RU486 (PR antagonist) did not block deactivation of MPN MOP and facilitation of lordosis by ARH infusion of OFQ/N. We confirmed that E2-GPER may act directly on ARH

OFQ/N neurons using double-label immunohistochemistry for GPER (Novus Biologicals) and OFQ/N (Neuromics), as well as GPER and ERα (Millipore), and ERα and OFQ/N. Subpopulations of ARH neurons were immunofluorescently double labeled for OFQ/N and GPER, OFQ/N and ERα, and GPER and ERα. These results indicate that E2 acts through GPER in OFQ/N neurons in the ARH to activate ORL-1 reducing β-END neuronal activity and facilitating lordosis. The colocalization of GPER and ERα indicate that estradiol can act through multiple ER mediated signaling pathways to regulate the activity of ARH neurons.

Disclosures: D.N. Tran: None. **N. Long:** None. **C. Serey:** None. **K. Sinchak:** None.

Poster

715. Estrogen Signaling and Behavior

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Title: Protein-protein interactions underly the interdependence of progesterone receptor-B, dopamine D1 receptor and Src family kinase signaling in the plasma membrane of the arcuate nucleus of the hypothalamus of female rats

Authors: *J. PHAN, D. LE, T. CHUON, K. SINCHAK;
Biol. Sci., California State University, Long Beach, Long Beach, CA

Abstract: Estradiol upregulation of classical progesterone receptor-B (PR-B) is necessary for progesterone facilitation of sexual receptivity (lordosis). Progesterone infused into the arcuate nucleus of the hypothalamus (ARH) facilitates lordosis within thirty minutes in ovariectomized (OVX) rats primed with 2μg of estradiol benzoate (EB). However, these rapid actions of progesterone indicate that it is signaling through extranuclear receptors to inhibit ARH β-endorphin neurons to induce lordosis. Although PR-B is a classical nuclear transcription factor, it is found in the cytoplasm and plasma membrane. We have shown PR-B to form complexes with Src family kinase (Src) in the cytoplasm and membrane cellular fractions from the ARH and that progesterone facilitation of lordosis requires Src activation. Progesterone facilitation of sexual receptivity is also mediated by the dopamine D1 receptor (D1). D1 has been hypothesized to

activate PR via a ligand independent mechanism (Mani et al., 1994; Mani et al., 1996). However, D1 can signal through multiple G protein signaling cascades as well as Src. We observed that PR, D1, and Src signaling pathways that facilitates sexual receptivity are interdependent. Antagonizing one, blocks facilitation of lordosis by the other two. This indicates that PR, D1, and Src signaling converge and are interdependent to facilitating sexual receptivity. We hypothesized that these PR-Src-D1 interactions are in ARH β-endorphin neurons. To test this, we performed double label immunohistochemistry in tissue from EB-primed female rats. Subpopulations of ARH β-endorphin immunopositive neurons also contained PR and D1 immunostaining. We showed previously PR and D1 colocalization in the ARH. Together, these results studies indicate the potential for expression of PR and D1 in β-endorphin neurons. Previously, we demonstrated that PR-B and D1 in ARH do not form complexes. Therefore, we tested the hypothesis that D1 and Src form complexes in the ARH. OVX rats were treated with EB or EB + progesterone and plasma membrane and cytosolic fractions were extracted from ARH block dissections. Western blot analysis revealed Src and D1 were present in the ARH, and co-immunoprecipitation experiments indicated the potential for D1 and Src to form complexes. Our results suggests that PR and D1 in the plasma membrane are capable of complexing with and signaling through Src in neurons of the ARH. These direct Src interactions with PR and D1 may explain the interdependence of these signaling proteins in the ARH to facilitate sexual receptivity.

Disclosures: **J. Phan:** None. **D. Le:** None. **T. Chuon:** None. **K. Sinchak:** None.

Poster

715. Estrogen Signaling and Behavior

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Topic: E.03. Behavioral Neuroendocrinology

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TL4U10009361

Title: Estradiol-GPER facilitation of lordosis in the female rat is via direct regulation of the orphanin FQ-ORL-1 system in the arcuate nucleus

Authors: *N. P. LONG, S. M. CHOKR, K. SINCHAK;
Biol. Sci., California State Univ. Long Beach, Long Beach, CA

Abstract: In the female rat, sexual receptivity (lordosis) can be facilitated by sequential activation of estrogen receptor (ER) α and G protein-coupled estrogen receptor 1 (GPER) by estradiol (Long et al., 2014, Horm Behav 66:63). Estradiol initially binds to ERα in the plasma membrane that complexes with and signals through metabotropic glutamate receptor 1a (mGluR1a) to rapidly activate β-endorphin (β-END) neurons in the arcuate nucleus of the hypothalamus (ARH) that project to the medial preoptic nucleus (MPN). This activates MPN μ-opioid receptors (MOP) to inhibit lordosis. Facilitation of lordosis is dependent on the subsequent deactivation of MPN MOP. In a 2 μg estradiol benzoate (EB) primed ovariectomized (OVX) rat, infusion of non-esterified 17β-estradiol (E2) 47.5 hours later reduces MPN MOP activation and facilitates lordosis in 30 minutes through activation of the orphanin FQ-opioid receptor-like-receptor-1 (ORL-1) system that reduces β-END neuronal activity. We previously demonstrated that lordosis can be facilitated by treating EB-primed OVX rats with selective estrogen receptor modulators (SERMs) tamoxifen (TAM) or ICI 182, 780 (ICI). Although TAM and ICI are classical estrogen receptor antagonists, they have also been reported to activate GPER. Therefore, we hypothesized that infusion of either TAM or ICI into the ARH of a 2 μg EB-primed rat will rapidly deactivate MPN MOP and facilitate lordosis through a GPER dependent pathway. Infusion of either TAM or ICI into the ARH 47.5 hours after EB priming facilitated sexual receptivity within 30 minutes and reduced MPN MOP activation compared to vehicle controls. Further, both the deactivation of MPN MOP and the facilitation of lordosis through infusion of TAM or ICI were significantly decreased when animals were pretreated with the GPER selective antagonist, G15. Our findings indicate that TAM and ICI act through GPER to deactivate MPN MOP and facilitate lordosis. Thus, these SERMs can activate GPER in the CNS to produce estrogenic actions in neural circuits that affect physiology and behavior.

Disclosures: N.P. Long: None. **S.M. Chokr:** None. **K. Sinchak:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.01/X30

Topic: E.03. Behavioral Neuroendocrinology

Title: Oxytocin receptors in the insular cortex mediate social affective behavior in rat

Authors: *M. M. ROGERS[1], A. F. PIERCE[2], J. P. CHRISTIANSON[2];
[1]Dept. of Psychology, [2]Boston Col., Chestnut Hill, MA

Abstract: Aberrant social behavior characterizes numerous psychiatric conditions like autism and schizophrenia. Impairments in the processing of affective information may account for deficits in empathy, perspective-taking and other social cognitions. The ability to detect the emotional state of an individual, or social affect, is a prerequisite for these sophisticated social behaviors. The insular cortex (IC) has rich sensory inputs and has been implicated in healthy and disordered social affect. Moreover, oxytocin (OT) modulates social behaviors and rich OT receptor (OTR) binding is found in the IC. To explore the contribution of insular OTR in social affect we introduced an affective manipulation to a rat social interaction test. In the Social Affective Preference (SAP) test an adult male rat is presented two unfamiliar male juvenile conspecifics, one exposed to stress (2x 5 s, 1mA footshocks) and the other naïve to treatment. In the SAP test adult male rats preferred to interact with the stressed juvenile ($p<0.01$). Bilateral infusion of OTR antagonist (10μg/side) to the IC prior to SAP testing abolished the preference for the stressed juvenile. The OTR is coupled to the extracellular signal-regulated kinases (ERK). Immunohistochemical analysis revealed that after a social interaction with a stressed juvenile, the number of phosphorylated ERK immunoreactive cells in the IC of adult male rats tended to correlate with time spent in interaction, but did not reach significance. Consistently, intra-insula inhibition of the ERK signaling cascade by U0126 (500ng/side) also prevented social affective preference. Together these data suggest that OTR and, possibly, ERK in the insular cortex are necessary for this form of social affect in rat.

Disclosures: M.M. Rogers: None. **A.F. Pierce:** None. **J.P. Christianson:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.02/X31

Topic: E.03. Behavioral Neuroendocrinology

Support: KAKEN # 23240057 to SO

KAKEN # 15H01844 to SO

Title: Effects of repeated presentation of social stimuli on social investigatory behavior in oxytocin receptor knockout male mice

Authors: *S. SAGOSHI[1], K. NISHIMORI[2], T. SAKAMOTO[3], S. OGAWA[1];
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Abstract: It is well established that oxytocin (OT) and oxytocin receptors (OTR) may play a primary role in the regulation of social recognition. However, it is still largely unknown about details in 'social amnesia' phenomenon (i.e., failure of discrimination between familiar and unfamiliar opponents) reported in OT and OTR knockout mice. In the present study, we aimed to determine whether repeated exposure to social stimuli might influence social investigatory behavior toward familiar and unfamiliar opponent mice in OTR knockout (OTRKO) male mice. Using social interaction test paradigm developed in our laboratory (SOSI Type1), we examined social investigatory (SI) behavior. Male OTRKO and their wild-type littermate (WT) mice were singly housed. Each mouse was tested for 4 days against a same male stimulus mouse presented in a transparent acrylic cylinder with holes placed at the center of his home cage for 4min per trial, 4 trials a day with 17min intervals. On Day 5, mice were tested similarly toward a novel male stimulus mouse. In each test, cumulative duration of sniffing toward stimulus mice was recorded as SI duration. We found WT mice showed a within-day decrease of SI duration toward the same stimulus starting on Day 1 whereas OTRKO mice failed to do so. However, staring on Day 3, OTRKO mice also showed a within-day decrease of SI duration. Moreover, on Day 5 when a novel stimulus was presented, both WT and OTRKO mice showed a clear within-day decrease of SI duration. These results suggest that repeated exposure to a stimulus mouse might be able to modify behavioral responses in social context in OTRKO mice and these effects of social experience might be generalized to their responses to a novel social stimulus. On the other hand, there was no spatial memory dysfunction in OTRKO mice in the Barnes maze task, suggesting that disruption of OTR gene affected behavioral responses specifically in social context.

Disclosures: **S. Sagoshi:** None. **K. Nishimori:** None. **T. Sakamoto:** None. **S. Ogawa:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.03/X32

Topic: E.03. Behavioral Neuroendocrinology

Support: R01 MH085069

Title: Effects of vasopressin V1a receptor in the bed nucleus of the stria terminalis on social withdrawal in males and females

Authors: *N. DUQUE, M. Q. STEINMAN, R. HAO, S. YOKOYAMA, B. C. TRAINOR; UC Davis, Davis, CA

Abstract: Oxytocin (OT) has important effects on social behaviors and coping responses to stress, with most studies indicating an anxiolytic effect of OT. However, in several cases OT has been reported to have anxiogenic effects. We recently discovered that social defeat stress induces hyperactivity in oxytocin neurons in the bed nucleus of the stria terminalis (BNST) and paraventricular nucleus, and that this effect is primarily observed in females but not males. These studies used the monogamous California mouse, which is one of the few species in which social defeat stress can be studied in both males and females. We found that intranasal infusions of OT reduced social interaction behavior in females but not males, which closely mirrors the effects of social defeat stress on this behavior in females. We also used autoradiography to quantify oxytocin receptor (OTR) and vasopressin V1a receptor (V1aR) expression. In the BNST, V1aR but not OTR was negatively correlated with social interaction behavior in females, and positively correlated in males. We used a pharmacological approach to test whether activation of V1aR in the BNST plays a role in stress-induced social withdrawal. Control and stressed males and females were randomly assigned to be infused with either artificial cerebrospinal fluid (CSF) or the selective V1aR antagonist β-Mercapto-β,β-cyclopentamethylenepropionyl1, O-me-Tyr2, Arg8]-Vasopressin). Stress reduced social interaction behavior in females and males. Intriguingly, V1A antagonist significantly reduced social interaction not only in naïve males, but also females, and had no effect on stressed animals. Thus, if hyperactivity of OT neurons contributes to social withdrawal in females, it appears that V1aR in the BNST are not an essential mechanism. Ongoing experiments are investigating whether other receptor populations contribute to stress-induced social withdrawal.

Disclosures: N. Duque: None. **M.Q. Steinman:** None. **R. Hao:** None. **S. Yokoyama:** None. **B.C. Trainor:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.04/X33

Topic: E.03. Behavioral Neuroendocrinology

Title: Examination of oxytocin and vasopressin on the three chamber sociability test in male or female mice injected flutamide or letrozole

Authors: *Y. MOMOTA[1], T. AMANO[3], H. KAWABATA[2], H. SAITOH[2];
[2]Akita Univ., [1]Sch. of Hlth. Sci., Akita, Japan; [3]Sch. of Hlth. Science, Akita Univ., Akita, Japan

Abstract: Oxytocin (OT) and Vasopressin (AVP) are participated in various neuronal activity like sexual behavior and social recognition. Estradiol (Est) and testosterone (T) are reported to regulate the expression of OT and AVP in the CNS. The present study examined the effect of OT and AVP on sociability to partner in the male and female mice administered flutamide (Flu) or letrozole (Let). Both male and female mice (ddy strain) were equally divided into 3 groups, that is, male or female vehicle mice (MV or FV), accordingly, flutamide administrated mice (MF or FF) and letrozole administrated mice (ML or FL). Let or Flu was administered po per day at the dose of 15mg/kg or 20mg /kg, respectively. Mice were examined the partner preference test which was assessed with three camber sociability test according to method by A.C. Felix-Ortiz and K. M.Tye. The apparatus was divided 3 chambers. Mini cages were placed in end chambers. In each session, test mouse selected from vehicle mice and drug administered mice was placed in center camber first. Then an unfamiliar different sex mouse (1st presenter) was in a mini cage in one of end chambers. Likewise, next another mouse (2nd presenter) was placed in another end chamber. Sociability to different sex was evaluated in staying time of test mouse in each end chambers located different sex mouse in last 10 min (ST). After initial test, effect of OXY (0.2 n mole per 10g bw, intranasal administraion) was examined in test mouse. One week later, the effect of AVP (0.2 n mole per 10 g b.w.) was estimated. Male mouse: Without OXY and VAP treatment, VM and MF mouse indicated significantly longer ST in 2nd presenter camber, however, the ST of ML mouse was not significantly different. OXY treatment to male 3 groups indicated longer ST in 2nd mouse chamber. VAP treatment to VM mouse showed as same as OXY treatment, however, MF and ML mouse showed significantly longer ST in 1st mouse chamber. Female mouse: Without OXY and VAP treatment, all test mouse showed significantly longer ST in t 2nd mouse chamber. OXY treatment to all female test mice showed no significantly different ST (that is, equally stayed in each end chamber). AVP treatment to VF and FL mouse did not show significantly difference in ST, however, FF mouse showed significantly longer ST in 2nd mouse chamber. From present results, female mice indicate high sociability to 2nd mouse, which is not affected by Est and T level. However, oxytocin treatment decreases the sociability. Form the result of VF mouse, AVP and OXY shows cross- reactivity in the sociability. The effect of OXY and AVP is roughly different between female and male mice. Male sociability to female mouse might be strongly influenced by action of estradiol and AVP.

Disclosures: **Y. Momota:** None. **T. Amano:** None. **H. Kawabata:** None. **H. Saitoh:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.05/X34

Topic: E.03. Behavioral Neuroendocrinology

Support: Florida State University

Title: Oxytocin receptor in the periphery of the perinatal mouse

Authors: R. VAIDYANATHAN[1], C. N. CARLTON[2], E. KIDWAI[2], T. A. MERRITT[2], I. SAKINAH[2], J. R. QUINTANA[2], G. G. HOFFMAN[1], *E. A. HAMMOCK[1];
[1]Psychology, [2]Florida State Univ., Tallahassee, FL

Abstract: Oxytocin (OXT) and the OXT receptor (OXTR) influence adult species-typical social behaviors. Peripheral OXT exposure during development leads to long term changes in the brain and in social behavior, but by unknown mechanisms that may involve OXT signaling through OXTR in the periphery. We previously observed OXTR ligand binding in peripheral tissues of the perinatal C57BL/6J mouse, including the mouth, nasal cavity, dermis, brown adipose tissue, adrenal gland, kidney, and anogenital area. This distribution of OXTR protein indicates that some of these peripheral OXT receptors are poised to detect maternal sources of OXT in milk, saliva, and amniotic fluid. To begin to refine our hypothesis of the potential role of maternal sources of OXT acting on peripheral OXTR, we have investigated the distribution of Oxtr mRNA by colorimetric *in situ* hybridization in the whole perinatal mouse with a probe specific to the 3'UTR of Oxtr mRNA. We observed abundant and widespread mRNA expression in the brain and the face. We detected modest levels of Oxtr mRNA in brown adipose tissue, adrenal glands, and kidneys. In all of these areas, most of the colorimetric detection was diffuse and did not have the characteristic appearance of mRNA in the cell body. In addition, we also observed significant Oxtr mRNA expression in the dorsal root ganglia and in the trigeminal nucleus. The staining in these ganglia was a characteristic mRNA profile localized in the cell body. This suggests an intriguing hypothesis that Oxtr mRNA is expressed by visceral sensory afferents (the dorsal root ganglia) and touch and pain afferents of the face (the trigeminal) and that those cells transport Oxtr mRNA to distal locations where we detected diffuse extra-somal pattern of mRNA. Trafficked mRNA may be locally translated into the observed OXTR protein in these peripheral tissues. These findings are consistent with our hypothesis that exogenous sources of OXT may modulate afferent sensory signals, and provide a hardwired circuit for maternal OXT to shape the experience-dependent development of the infant brain.

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Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.06/X35

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH R21-AA020575

Title: Prolactin decreases social avoidance and is required for short-term social memory

Authors: *M. DONHOFFNER[1], R. I. WOOD[2];
[1]USC, Los Angeles, CA; [2]Cell and Neurobio., Keck Sch. of Med. of the Univ. of Southern California, Los Angeles, CA

Abstract: Prolactin (PRL) is an anterior pituitary hormone essential for milk production during lactation. Oxytocin (OT) is a posterior pituitary hormone essential for milk-let down during lactation, but also functions as a neuromodulator to promote affiliative behaviors such as social memory, and approach. Since PRL and OT have complementary peripheral functions in lactation it is reasonable to expect that they share similar central functions to promote social behavior. Accordingly, we hypothesized that PRL increases social approach and social memory. To test this hypothesis, we determined if PRL restores approach behavior following acute social defeat. We also tested whether PRL increases discrimination of a familiar vs a novel rat. Male Long-Evans rats were injected ip with ovine PRL (2 mg/kg), the PRL antagonist bromocriptine (3 mg/kg) or saline vehicle 20 mins prior to behavioral testing. For social defeat, the test rat was placed in the home cage of a larger, aggressive male for 30 mins; non-defeated controls were placed in an empty cage. 2 hours later, rats were tested for 4 mins for approach (within 4 cm) towards an empty cage, followed by approach towards an identical cage containing the defeater rat. Compared to non-defeated controls, defeated rats avoided the defeater, but there was no difference in approach towards the empty cage. PRL administration, either before or after social defeat, restored approach towards the defeater rat (PRL before: 167.9±6.5 sec/4mins, PRL after: 154.4±11.1, defeated vehicle 107.3±6.6, $p<0.005$). In non-defeated rats, treatment with PRL or bromocriptine had no effect. These results suggest that PRL increases social approach in defeated rats. To determine whether PRL increases social memory, we tested rats with a social discrimination task. The test rat was housed with an unfamiliar stimulus rat for 4 mins. After 45 mins (short-term memory) or 2 hr (long-term memory), the test rat was exposed to both the familiar rat and a novel stimulus rat for 4 mins, and the time spent investigating each rat was measured. Rats prefer a novel rat over a familiar stimulus animal, and this discrimination persists for ca. 1 hr. After a 45-min delay, rats treated with bromocriptine to reduce PRL failed to show a

preference for the novel rat (familiar: 57.5±9.9sec vs novel: 61.4±8.2, p>0.05). However, PRL treatment did not enhance preference for the novel rat after a 2 hr delay (familiar: 56.1±6.3sec vs novel: 54.3±9.3, p>0.05), similar to saline-treated controls. These results suggest that PRL is required for short-term social memory. Therefore, this study suggests that PRL promotes affiliative social behaviors, similar to OT.

Disclosures: M. Donhoffner: None. **R.I. Wood:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.07/X36

Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC Grant 05388

Title: Effects of intranasal and intraperitoneal oxytocin administration on social interaction and hypothalamic-pituitary-adrenal activity in rats

Authors: *P. KENT[1], A. AWADIA[2], D. ENSAN[2], L. ZHAO[3], D. SILVA[2], C. CAYER[2], Z. MERALI[2];
[1]Uottawa Inst. of Mental Hlth. Res., Ottawa, ON, Canada; [2]Univ. of Ottawa Inst. of Mental Hlth. Res., Ottawa, ON, Canada; [3]Mcgill Univ., Ottawa, ON, Canada

Abstract: Oxytocin is a nine amino acid neuropeptide associated with prosocial behaviour in healthy subjects. It has been suggested that oxytocin's ability to increase sociability is through a reduction in stress reactivity. In light of its prosocial effects, oxytocin is increasingly being recognized as a novel therapeutic target for psychiatric disorders characterized by social dysfunction including social anxiety, schizophrenia and autism. Owing to poor blood brain permeability, oxytocin is usually administered intranasally in humans as this route is thought to provide direct access to the brain. There are however, surprisingly few preclinical studies investigating effects of intranasal oxytocin in rodents which should be pursued given that intranasal oxytocin is increasingly being prescribed by health practitioners. With this in mind, in the present study, we assessed the effects of intranasal oxytocin on social interaction and release of corticosterone (a measure of stress reactivity) in rats and compare these effects with those elicited by the more traditional intraperitoneal route of administration. Intranasal and systemic administration of 20 but not 5 μg of oxytocin elicited a significant increase in social interaction. In addition, while intranasal oxytocin (20 μg) had no effect on blood corticosterone levels, a

marked increase in blood corticosterone levels was observed following systemic oxytocin administration. These findings in rodents are consistent with those observed in humans in that intranasal oxytocin increases prosocial behaviours. However, these behavioural effects appear unrelated to corticosterone levels refuting suggestions that the prosocial effects of oxytocin stem from reduced stress reactivity.

Disclosures: **P. Kent:** None. **A. Awadia:** None. **D. Ensan:** None. **L. Zhao:** None. **D. Silva:** None. **C. Cayer:** None. **Z. Merali:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.08/X37

Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC Graduate Scholarship

Title: Arginine vasopressin influences the social behavior of free-living Richardson's ground squirrels (Urocitellus richardsonii)

Authors: A. R. FREEMAN[1], J. F. HARE[2], G. ANDERSON[2], *H. K. CALDWELL[1];
[1]Dept. of Biol. Sci., Kent State Univ., Kent, OH; [2]Biol. Sci., Univ. of Manitoba, Manitoba, MB, Canada

Abstract: In many vertebrate taxa arginine vasopressin (Avp) and its homologues are important to the neural regulation of social behaviors. In rodents, Avp is best known for its modulation of affiliative behaviors such as grooming, sniffing, and the formation of social bonds and memories. Avp is also important for species-specific vocalizations, as demonstrated through work on birds, fish, and amphibians. More recently, Avp has been found to influence social communication in laboratory rodents (i.e. rat and mouse) by altering pup ultrasonic vocalizations. However, this work has not been extended to other rodent species or to free-living individuals of any species. Richardson's ground squirrels (*Urocitellus richardsonii*) are free-living social rodents, in which alarm calling serves as a proximate manifestation of sociality. In order to determine how Avp effects social communication in the wild, we implanted osmotic minipumps into Richardson's ground squirrels and administered Avp or saline intracerebroventricularly. We then examined behavior before and after Avp or saline administration using three assays: 1) a general behavior survey, 2) a predator model presentation, and 3) a social challenge experiment. While saline had no effect, Avp reduced aggression and increased antipredator vigilance and escape behavior in

males, but had no effect on the propensity to emit alarm calls in response to a predator model. However, during the social challenge, Avp-treated males increased chirp-type vocalizations during social interactions. These context-specific effects (i.e. predator versus conspecific) on communication are interesting and mirror effects seen in olfaction experiments with laboratory rodents. This work is significant as it is the first of its kind to examine the effects of Avp on social communication in the wild. Further, our discovery of Avp's effects on vigilance in a social setting is particularly exciting and highlights Avp's extensive influence on social behavior.

Disclosures: A.R. Freeman: None. **J.F. Hare:** None. **G. Anderson:** None. **H.K. Caldwell:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.09/X38

Topic: E.03. Behavioral Neuroendocrinology

Support: Indiana University CISAB Summer Fellowship

Title: Sex-specific influence of vasotocin cell groups on behavioral phenotype and phasic response to social contact in a gregarious finch

Authors: *C. L. PETERSEN, S. E. SCHROCK, M. A. KINGSBURY, J. L. GOODSON; Evolution, Ecology and Behavior, Indiana Univ., Bloomington, IN

Abstract: Sociality (grouping behavior) is but one aspect of an animal's social-behavioral repertoire. In estrildid finches, species-specific diversity in sociality is mediated by the nonapeptides arginine vasotocin (VT; Ile^3-Vasopressin) and mesotocin (MT; Ile^8-Oxytocin). Likewise, how individual finches respond to social or stressful conditions i.e., their behavioral phenotype or "personality" is significantly related to variation in constitutive expression of VT and MT in multiple brain regions. Less is known, however, about the relationship between measures of "personality" and how these nonapeptide groups respond to social contact, a metric of sociality. Here we test the hypothesis that behavioral phenotype will influence patterns of neural activation in response to social contact within multiple VT populations in a sex-specific manner. Male and female zebra finches (*Taeniopygia guttata*) were behaviorally phenotyped to determine individual levels of social contact time (sociality) and anxiety-like behaviors. Next, finches were housed overnight in sound attenuation booths in visual isolation from conspecifics. The next morning, focal birds had 2 novel same-sex conspecifics placed in their cages. Control

birds were left in social isolation. After 90 minutes, birds were sacrificed and brains were processed for multi-fluorescent immunocytochemistry (ICC). We investigated labeling of the immediate early gene product FOS within VT neurons in the medial bed nucleus of the stria terminalis (BSTm) in the amygdala, and the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. We find that males exposed to same-sex conspecifics have a significantly higher percentage of VT-FOS colabled neurons in the BSTm than do control males. While there is no difference in VT-FOS colocalization in the BSTm of females, there is a significant correlation between social contact time (during personality assessment) and VT-FOS co-labeled cells in BSTm in both sexes. We find no difference in VT-FOS colocalization in the PVN for either sex or social treatment condition. Within the SON as a whole, there was no difference in VT-FOS colabeling in males or females regardless of condition; however, females had higher levels of VT-FOS colabeled neurons in the medial SON than males regardless of treatment group. Finally, total number of VT SON neurons correlated positively with social contact time in males, but not in females. To our knowledge, these data are the first to demonstrate how constitutive levels of VT in SON can relate to measures of an animal's "personality", and they offer a more complete picture of VT activation during social contact.

Disclosures: C.L. Petersen: None. **S.E. Schrock:** None. **M.A. Kingsbury:** None. **J.L. Goodson:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.10/X39

Topic: F.03. Motivation and Emotion

Support: Grants-in-Aid for Science Research on Innovative Areas, "The Science of Mental Time" (25119004)

Title: Experience-induced facilitation of mouse empathetic behavior

Authors: *T. SAKAGUCHI, S. IWASAKI, K. OKAMOTO, Y. IKEGAYA;
Pharmaceut. Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: Empathy, a high-level cognitive process, is believed to exist exclusively in humans; however, recent evidence has demonstrated empathy-like behaviors in rodents. The rodent models provide experimental platforms to investigate the neural basis that brings about empathy. We developed a fear observational system in which a mouse (observer) exhibits freezing

behavior through observation of another freezing mouse (demonstrator) that receives repetitive foot shocks. We found that observers showed higher freezing responses when they had received a priming foot shock. The priming shock could not be replaced with other aversive experiences, such as forced swimming and tail pinch, which suggests that empathy is facilitated by a common, shared experience. Moreover, the priming shock-induced increase in freezing time was abolished by systemic injection of MK801, an NMDA receptor antagonist. Thus, the priming effect is likely to emerge through NMDA receptor-dependent neuronal plasticity. We then focused on oxytocin, which is known to have broad effects on social behaviors in humans as well as rodents. Intraperitoneal injection of L-368,899, an oxytocin receptor antagonist, 30 min prior to fear observation attenuated the freezing response. Thus, oxytocin is likely to mediate the experience-dependent modulation of empathetic behaviors of fear-observing mice. Next, we investigated whether the priming shock leads to a reorganization of neuronal ensembles in the neocortices. To identify neurons that were active during the priming shock and/or the fear observation, we used temporal activity mapping with cellular resolution by detecting *Arc* mRNA (i.e., catFISH). We revealed that the neuronal ensembles that were active during the priming shock were significantly overlapped with those that were active during the fear observation, suggesting that the same neuron subpopulation was recruited in firsthand and vicarious pain representation. Thus, empathy may be a vicarious experience of what another person feels at the neuron level.

Disclosures: **T. Sakaguchi:** None. **S. Iwasaki:** None. **K. Okamoto:** None. **Y. Ikegaya:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.11/X40

Topic: E.03. Behavioral Neuroendocrinology

Title: Molecular mechanisms underlying sex differences in the brain oxytocin system

Authors: *N. B. WORLEY;
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Abstract: The neuropeptide oxytocin (OT) has been shown to modulate social behaviors, and often does this in sex-specific ways. This may be due to sex differences in the brain OT system. In support, our lab has recently demonstrated that adult male rats have higher OT receptor (OTR) binding densities than females in various forebrain regions, particularly in the posterior bed nucleus of the stria terminalis (BNSTp) and the ventromedial hypothalamus (VMH). Here, we

have started to explore the molecular mechanisms underlying the sex differences in OTR binding. We show that the sex difference in OTR binding density is present before and after puberty in the BNSTp, but only after puberty in the VMH. These sex differences, or lack thereof, correspond with a sex difference in OTR mRNA expression. From this, we hypothesized that the sex difference in OTR mRNA expression in the pBNST, but not the VMH, is the result of gonadal steroid hormone-dependent chromatin remodeling during early postnatal life. Specifically, we predicted that histone deactylation in early postnatal life plays a role in sexual differentiation of OTR mRNA expression in the BNSTp. However, we find that neonatal treatment with a histone deacetylase inhibitor did not alter OTR binding density in the BNSTp or VMH in either sex. Overall, these findings suggest that (1) the sex differences in OTR binding density in the BNSTp and VMH are due to a sex difference in OT mRNA expression, (2) the age of onset of sex differences in OTR is brain region-specific, suggesting separate, or at least temporally disjointed, underlying mechanisms, and (3) the sex difference in OTR binding in the BNSTp may not be the result of a sex difference in histone deacetylase activity in the neonatal rat.

Disclosures: N.B. Worley: None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS1253386

NSF GRFP 2012138127

Title: Sub region-specific distribution of μ-opioid receptors in the striatum of juvenile rats: Implications for social novelty preference

Authors: *C. J. SMITH, A. M. RATNASEELAN, M. L. POEHLMANN, A. H. VEENEMA; Boston Col., Chestnut Hill, MA

Abstract: The drive to approach and explore novel conspecifics is inherent to social animals and may promote optimal social functioning. Juvenile animals seek out interactions with novel peers more frequently and find these interactions to be more rewarding than their adult counterparts. We have previously shown that male and female juvenile rats spend more time interacting with a

novel conspecific than a cage mate. However, the neural systems regulating this social novelty preference have yet to be elucidated. We hypothesized that brain systems subserving social information processing and social motivation/reward, i.e., the opioid, dopamine, oxytocin, vasopressin systems, might support social novelty preference. To test this, we used intracerebroventricular antagonist administration to block receptors of each of these systems prior to social novelty preference testing. Central blockade of μ-opioid receptors (MORs) reduced both the duration and frequency of novel social interaction while leaving interaction with the cage mate unaffected. In contrast, central blockade of dopamine D2, oxytocin, or vasopressin V1a receptors failed to alter social novelty preference. Given these results, we next asked where in the brain MORs act to support social novelty interaction, and to that end, where in the brain MOR binding densities are highest in juvenile rats. Based on MOR binding patterns, we analyzed MOR binding density in six subregions of the striatum, namely the anterior caudate putamen (CP), posterior CP, nucleus accumbens (NAc) core, and anterior, dorsomedial, and ventral NAc shell. MOR binding density was the highest in the anterior CP compared to all other subregions. Additionally, MOR binding density within NAc subregions was the highest in the dorsomedial NAc shell. We are currently investigating the role of MORs in these two striatal subregions in the regulation of juvenile social novelty preference. Previous studies suggest a role for the NAc but not the CP in regulating the rewarding aspects of social novelty interaction. Therefore, we will test the hypothesis that MORs in the dorsomedial NAc shell, but not in the anterior CP, facilitate social novelty preference. Understanding the role of the MOR system in social novelty seeking may help to elucidate the neural mechanisms underlying abnormalities in this behavior, as seen in autism and substance abuse disorders. This research was supported by NSF graduate research fellowship to CJS 2012138127 and NSF IOS1253386 to AHV

Disclosures: C.J. Smith: None. **A.M. Ratnaseelan:** None. **M.L. Poehlmann:** None. **A.H. Veenema:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

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Program#/Poster#: 716.13/X42

Topic: E.03. Behavioral Neuroendocrinology

Support: NARSAD Grant 17382

NSF IOS1253386

NIMH R01MH102456

Title: Vasopressin regulates social play in sex-specific ways through glutamate modulation in the lateral septum

Authors: *R. BREDEWOLD, J. K. SCHIAVO, M. VERREIJ, G. RO, A. H. VEENEMA; Dept. of Psychology, Boston Col., Chestnut Hill, MA

Abstract: Social play is an affiliative and rewarding behavior displayed by nearly all mammals and peaks during the juvenile period. We recently showed that arginine vasopressin (AVP) acting via the V1a receptor (V1aR) within the lateral septum (LS) regulates social play in opposite directions in male and female juvenile rats. Specifically, administration of the specific V1aR antagonist (CH2)5Tyr(Me2)AVP into the LS decreased social play in females and increased social play in males . Because previous *in vitro* studies suggest that AVP modulates glutamate and GABA responses in the LS, we hypothesized that AVP may regulate social play differently in males and females by sex-specific modulation of glutamate and/or GABA neurotransmission. Using retrodialysis combined with microdialysis in awake and freely moving juvenile rats, we found that V1aR blockade in the LS increased the release of glutamate within the LS in females but not in males, while GABA release was increased in both sexes. We next determined whether the increase in glutamate release in females underlies the V1aR-induced decrease in social play. Administration of the glutamate receptor agonist, L-glutamic acid, prior to the social play test, decreased social play in females thereby mimicking the behavioral effects of V1aR blockade. This decrease in social play could also be induced in males by injecting L-glutamic acid in the LS. Moreover, L-glutamic acid prevented the V1aR antagonist-induced increase in social play behavior in males. In conclusion, an increase in glutamate in the LS, prior to the play test, has an inhibitory effect on social play in both males and females. These findings further suggest that the sex-specific regulation of social play by AVP involves differential glutamate neurotransmission in the LS of male and female juvenile rats.

Disclosures: R. Bredewold: None. **J.K. Schiavo:** None. **M. Verreij:** None. **G. Ro:** None. **A.H. Veenema:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIMH Grant R15MH102807

Title: Dynamic vasopressin release in the lateral septum during social recognition in adult and juvenile male and female rats

Authors: *B. DIBENEDICTIS, R. BREDEWOLD, A. H. VEENEMA;
Psychology Dept., Boston Col., Chestnut Hill, MA

Abstract: Vasopressin (VP) is a neuropeptide implicated in the regulation of a myriad of social behaviors. VP signaling transpires within a circuit of interconnected limbic structures known collectively as the 'social behavior neural network.' In both rodents and humans, VP signaling has been associated with the regulation of social recognition (the ability to discriminate between familiar and unfamiliar individuals), a behavior critical for appropriate and adaptive social interactions. The lateral septum (LS) receives sexually dimorphic (more in adult males) VP fiber innervation. However, the extent to which sex differences in static LS-VP fiber density correlate with dynamic changes in VP release during social interactions remain unknown. Previous work from our laboratory demonstrated that VP signaling within the LS differentially regulates social recognition in adult versus juvenile rats and in males versus females. We hypothesized that differential LS-VP release in juvenile and adult males and females underlies sex and age-specific regulation of social recognition by VP and that increased VP release is positively correlated with a greater density of VP fibers in the LS. To test this, we are utilizing intracerebral microdialysis in the LS to measure VP release during social recognition in adult and juvenile rats of both sexes. Additionally, we are employing VP immunohistochemistry to determine the relationship between VP fiber density and VP release within the LS. Preliminary results indicate that while adults possess a greater density of LS VP-IR fibers than juveniles, all groups show similar levels of baseline VP release. This work will reveal for the first time how sex and age differences in LS-VP innervation relate to dynamic VP release patterns during social behavior.

Disclosures: B. Dibenedictis: None. **R. Bredewold:** None. **A.H. Veenema:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS1253386

NIMH R01MH102456

NIMH R00MH093412

Title: Vasopressin modulates lateral septum neuronal activity in sex-specific ways in juvenile rats

Authors: *A. H. VEENEMA, R. BREDEWOLD, J. VARELA, J. P. CHRISTIANSON; Dept. of Psychology, Boston Col., Chestnut Hill, MA

Abstract: We recently showed that arginine vasopressin (AVP) regulates social play behavior in sex-specific ways in juvenile rats. Specifically, blockade of the AVP V1a receptor (V1aR) within the lateral septum (LS) enhanced social play in males, but reduced social play in females. Here, we sought to reveal the underlying mechanisms. Because *in vitro* studies suggest that AVP modulates glutamate and GABA responses in the LS, we determined whether AVP administration into the LS modulates the *in vivo* release of glutamate and GABA and the *in vitro* frequency of spontaneous excitatory post synaptic currents (sEPSC) differently in male and female juvenile rats. By combining retrodialysis with microdialysis in awake and freely moving rats, we found that AVP increased extracellular LS-glutamate in females, while decreasing it in males. AVP did not alter extracellular LS-GABA in either sex. Moreover, baseline extracellular glutamate, but not GABA, was higher in males than in females, a sex difference that was abolished after AVP administration. In whole-cell voltage clamp recordings from acute LS slices from either male or female juveniles, AVP appeared to reduce the number of sEPSCs in males (greater inter-event-interval, 4 of 6 cells) whereas AVP did not appear to alter sEPSCs in females (no changes observed in 3 of 4 cells) indicating sex differences in the modulation of glutamatergic synaptic transmission in the LS; data collection is ongoing. Together, these findings suggest that the sex-specific regulation of social play by AVP may involve differential glutamate signaling in the LS of male and female juvenile rats.

Disclosures: A.H. Veenema: None. **R. Bredewold:** None. **J. Varela:** None. **J.P. Christianson:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

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Program#/Poster#: 716.16/X45

Topic: F.01. Human Cognition and Behavior

Support: NRF-2010-0018837

NRF-2010-0012185

SK Telecom

Title: Emotion reading with eye tracker

Authors: *S. PARK, S. NAM, H. CHOI, D.-S. KIM;
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Abstract: Spontaneous recognition of user emotion with passive sensors which measure the user's physical state or behavior has been widely studied. For example, Pupil response, heart rate, galvanic skin response or skin temperature are some of the most highly captured physical data to determine user emotional state. In addition, gaze trajectory, facial expression or body posture are also the most useful user behavior data obtained from video camera to determine emotion. In the current study, we proposed a simple emotion reading system using eye-tracking glasses which has one infrared camera to record pupil diameter and position while the other camera record user view point in order to estimate user pupil response on the certain gaze trajectory point. Seven healthy volunteers (seven males, age 24±3) participated in the study. Each participant was presented totally 60 pleasant & excited, 60 pleasant & calm, 60 unpleasant & excited, and 60 unpleasant & calm during whole experiment while wearing eye-tracking glasses. The pictures were selected from the International Affective Picture System (IAPS) database (Lang et al., 2008) based on the IAPS's valence (1 is unpleasant and 9 is pleasant) and arousal (1 is calm and 9 is excited) standard scores of male. Therefore, only male subjects were selected due to expected sex differences in certain images. All images were adjusted to be a gray-scale and the mean luminance of 107. The experiment consisted of four sessions, and one session consisted of 60 trials. Cross fixation of 3 sec, picture stimuli of 6 sec, response and rest for 6 sec were sequentially presented in each trial. The subjects were asked to experience any feelings which the pictures might arouse in them, and rate each picture in a 2-point valence scale and a 2-point arousal scale using a keyboard during response time. All subjects were also asked not to move during a session and not to blink during cross fixation and picture stimuli. The time series of pupil diameter changes while the subject gazed at a picture were considered to classify valence or arousal, which mean pleasant vs unpleasant or calm vs excited, respectively. Certain time points which reject the null hypothesis from a paired t-test were selected for the features to classify. The valence classification in the session 3 of subject 6 showed the highest score of 76.9% accuracy while the best true positive result was 87.5% and the best true negative result was 100%. The arousal classification in the session 4 of subject 6 showed the highest score of 80.0% accuracy while the best true positive or true negative was 100%. This result showed the possibility of emotion reading system using eye-tracking glasses.

Disclosures: **S. Park:** None. **S. Nam:** None. **H. Choi:** None. **D. Kim:** None.

Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 716.17/X46

Topic: F.03. Motivation and Emotion

Title: Analysis of neural/molecular mechanisms of mate-guarding behavior in small fish, medaka

Authors: *S. YOKOI[1,2], T. OKUYAMA[1], Y. KAMEI[2], K. NARUSE[2], Y. TANIGUCHI[3], S. ANSAI[4], M. KINOSHITA[4], L. J. YOUNG[5], N. TAKEMORI[6], T. KUBO[1], H. TAKEUCHI[1,7]; [1]Univ. of Tokyo, Tokyo, Japan; [2]Natl. Inst. for Basic Biol., Okazaki, Japan; [3]Kyorin Univ., Tokyo, Japan; [4]Kyoto Univ., Kyoto, Japan; [5]Emory Univ., Atlanta, GA; [6]Ehime Univ., Toon, Japan; [7]Okayama Univ., Okayama, Japan

Abstract: In various animal species from insects to vertebrates, males exhibit mate-guarding behavior to prevent other males from mating a potential or former mate, which is one of the forms of male-male competition for mates. Although the mate-guarding behavior has been studied extensively in behavioral ecology and phylogeny, its neural/ molecular basis is unknown. We previously demonstrated that medaka (Oryzias latipes) fish, which is a model animal for molecular genetics, exhibited mate-guarding behavior. In addition, we also showed that vasotocin (non-mammalian homolog of vasopressin) might have a role of enhancing this behavior by using pharmacologic methods. In the present study, we report that we generated mutants of vasotocin and its receptors (V1a1 and V1a2) genes by TILLING methods and revealed that two genes, vasotocin and V1a2, are required for normal mate-guarding behavior. In addition, behavioral analysis of courtship behaviors in a dyadic relationship and aggressive behaviors within a male group revealed that vasotocin mutant males displayed decreased sexual motivation but showed normal aggression. In contrast, heterozygote V1a2 mutant males displayed decreased aggression, but normal mate-guarding and courtship behavior. Therefore, impaired mate-guarding in vasotocin homozygote mutants may be due to the loss of sexual motivation toward the opposite sex, and not to the loss of competitive motivation toward rival males. The different behavioral phenotypes between avt, V1a2 heterozygote, and V1a2 homozygote mutants suggest that there are redundant systems to activate V1a2 and that endogenous ligands activating the receptor may differ according to the social context.

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Poster

716. Social Behavior: Oxytocin and Vasopressin

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Topic: F.03. Motivation and Emotion

Support: Ministry of Education, Culture, Sports, Science and Technology of Japan

Tohoku leading women's Jump up project

Title: Generation of Oxtr cDNAHA-Ires-Cre mice for gene expression with oxytocin receptor specific manner

Authors: *S. HIDEMA[1], Y. HIRAOKA[1], H. MIZUKAMI[3], T. FUKUDA[2], S. SUZUKI[1], A. OTSUKA[1], S. MIYAZAKI[1], K. NISHIMORI[1];
[1]Mol. Biol., [2]Animal Breeding and Genet., Grad. Sch. of Agr. Sciences/Tohoku Un, Sendai-Shi, Japan; [3]Ctr. for Mol. Med., Jichi Med. University,, Shimotsuke, Japan

Abstract: The neurohypophysial hormone oxytocin (OXT) and its receptor (oxytocin receptor, OXTR) have a critical role in the regulation of pro-social behaviors, defined as voluntary behaviors intended to benefit another, such as social recognition, pair bonding or parental behaviors (Neumann, 2008). We previously generated knockout mice for Oxt (Nishimori 1996) and Oxtr (Takayanagi, 2005). The OXT ligand and Oxtr deficient mice showed abnormalities in various pro-social actions, such as social, maternal behaviors and social buffering (Smith and Wang, 2014). In order to visualize the distribution of OXTR in the central nervous system (CNS) in mice, we established an OXTR-Venus knock-in mice (Yoshida, 2009). Our established Oxtr-Venus knock-in mice allowed us to identify the detailed distribution of the OXTR. These knock-in mice express enhanced yellow-fluorescent protein (EYFP; Venus) in various types of cells and tissues in the CNS, indicating the endogenous expression of OXTR in the positive tissues and cells. Interestingly, we detected nuclei-restricted expression of Venus in various regions of the brain, including the amygdaloid body, medial preoptic area, and raphe nucleus, among others. These areas are potentially associated with the regulation of social and sexual behaviors through OXT and OXTR signaling. In the present study, we established Oxtr cDNAHA-Ires-Cre knock-in mice expressing both the OXTR and Cre recombinase under the control of the endogenous Oxtr gene promoter. We demonstrate that these mice allow specific gene expression in a Cre recombinase dependent manner in OXTR-expressing neurons, with infection of recombinant adeno-associated virus including flip-excision switch (AAV-FLEX) vector that carried irreversible type loxP sequences. The social behavior of Oxtr cDNAHA-Ires-Cre knock-in mice

was found to be the same as that of wild-type animals. In addition, the distribution of Cre expression was the same as that observed in Oxtr-Venus mice. Furthermore, we showed restricted expression of the tracing molecule wheat-germ agglutinin with the fluorescent protein mCherry in OXTR-expressing neurons by nuclei-specific infection of recombinant AAV-FLEX including inverted WGA-2A-mCherry sequence in these knock-in mice. This study could contribute toward monosynaptic analysis of neural circuits and optogenetic analysis of neurons expressing OXTR. Our established knock-in mouse would be a powerful tool for the functional analysis of oxytocin-positive neurons in social behaviors.

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Poster

716. Social Behavior: Oxytocin and Vasopressin

Location: Hall A

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Topic: E.03. Behavioral Neuroendocrinology

Support: NICHD 5T32HD007151

Title: Intranasal oxytocin enhances socially transmitted fear behavior in mice

Authors: ***M. T. PISANSKY**[1], J. C. GEWIRTZ[2];
[1]Neurosci., [2]Psychology, Univ. of Minnesota, Minneapolis, MN

Abstract: Oxytocin is an evolutionarily conserved neuropeptide implicated in social cognition processes. The oxytocin system is dysregulated in psychiatric diseases characterized by impaired social cognition, and intranasal oxytocin enhances emotional recognition (a component of "empathy") in both normal and psychiatric populations. In order to investigate the neurobiological effects of oxytocin on social cognition, we have developed a measure of socially transmitted fear, in which an observer mouse views a demonstrator mouse undergoing Pavlovian fear conditioning. Observer mice exhibit freezing, and - importantly - this effect occurs more robustly when the observer-demonstrator pair are familiar (i.e., siblings) with one another. We hypothesized that oxytocin would enhance socially transmitted fear. Therefore we administered oxytocin intranasally (20ug/kg) to non-sibling observer mice using a sub-chronic regimen (5 days). Compared to saline controls, oxytocin-treated observer mice exhibited significantly more freezing during the conditioning of demonstrator mice. Observer freezing behavior also correlated with demonstrator distress vocalizations recorded during conditioning, but only for

sibling and oxytocin-treated non-sibling observer mice. Interestingly, the enhanced freezing effect was not seen if oxytocin was administered using a single dose 30mins prior to testing. This suggests that sub-chronic intranasal oxytocin produced changes in endogenous oxytocin signaling mechanisms. To test this hypothesis, we are conducting immunohistochemical and quantitative PCR analyses of oxytocin receptor expression in observer mouse brain tissue. These experiments promise to elucidate the neurobiological effects of oxytocin on social cognition processes and contribute to our understanding of psychiatric diseases in which these processes are compromised.

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Poster

717. Social Behavior

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Program#/Poster#: 717.01/Y1

Topic: F.03. Motivation and Emotion

Title: A history of enrichment alters the outcome of a social preference task in adolescent rats

Authors: *K. L. PATTERSON, H. L. JOHNSON, E. A. ARTZ, O. W. FIELDS, R. GUCWA, D. I. ALEWEL, M. C. ZRULL;
Appalachian State Univ., Boone, NC

Abstract: The physical, social, and cognitive development of adolescents is often accompanied by behavior changes including increased interest in novelty and risk taking. For rats, the changes involve not only how adolescents explore objects in the environment but also how they interact with conspecifics. Social preference (SP) offers a measure of novelty seeking behavior relevant for study in adolescent animals. Environmental enrichment (EE), exposing an animal to a novel and enhanced environment on a regular basis, can modulate these behaviors. Over time, EE can allow rats to exhibit more flexible behaviors when placed in novel situations. We investigated the impact of EE on SP in adolescent rats. Long Evans rats (n = 12) were exposed to enrichment cages in same-sex groups for 1.5-h 18 times between postnatal days (pnd) 21 and 49. Age matched controls (n = 13) were left alone, aside from being briefly held 18 times. On pnd 35 and pnd 49, rats performed a SP task, which involved placing a rat into a small cage with additional small cages on either side of the experimental rat that each contained a stimulus rat. The time the experimental rat spent contacting the wire mesh wall between it and each stimulus rat as well as nose pokes at stimulus rats were measured during a 3-min trial. Then, after a 30-min delay, one stimulus rat was replaced with a novel stimulus rat and another trial was completed. After

testing, brain tissue was processed to examine levels of neural activity in amygdala evoked by the SP task using immunohistochemistry for c-fos. At pnd 35, the proportion of the 180-s Trial 2 time spent socializing with either stimulus rat by EE and control rats was similar; however, at pnd 49, the proportion decreased by 43% for EE and only by 10% for control rats ($p < .01$). Across pnd 35 and 49 test sessions on the delay trial, EE rats spent a similar proportion of time near the known and novel stimulus rat ($M = 0.53$, $SD = 0.13$) while control rats spent a greater proportion of time near the novel stimulus rat ($M = 0.65$, $SD = 0.15$) ($p < .02$). While EE rats decreased Trial 2 nose pokes at the novel stimulus rat between tests at pnd 35 and 49 (-12%), controls rats increased nose pokes at the novel conspecific by 12% ($p < .05$). Behavioral data suggest that periodic exposure to enriched environments throughout adolescence promotes fluent adaptation to social novelty with EE rats exhibiting more balanced attempts at contact with known and novel conspecifics than rats without EE history. If preference for an unknown over a known conspecific can be considered risky, then EE seems to suppress this type of risk taking behavior in our rat model.

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Poster

717. Social Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: OMHF Grant 051449

Title: The effects of a hyperandrogenic prenatal environment on early and later-life social behaviour

Authors: *C. S. WASSON[1], C. HOWES[1], M. CASTRO[2], A. SMART[2], A. J. GIUGA[2], N. J. MACLUSKY[3], E. CHOLERIS[1];
[1]Psychology and Collaborative Neurosci. Program, [3]Biomed. Sci., [2]Univ. of Guelph, Guelph, ON, Canada

Abstract: Testosterone drives the organization and development of the typical male phenotype, including sexually dimorphic behaviors. The developmental effects occur during the pre- and post-natal period to preadolescence. Subsequently, hormones have activational effects on sexually dimorphic behaviors throughout adult life. Most behavioral research has focused on

activational effects, leaving the developmental effects less understood. In particular, the developmental effects of testosterone on social behaviors, such as social approach, recognition and interactions, are not well elucidated. A female advantage in estrogen-dependent social behaviors is typically observed. In turn, “maleness” is often associated with aggression and asociability, with both testosterone and its metabolites being involved. Here we treated pregnant CD1 mice with 10μg of testosterone propionate systemically during embryonic days 12, 14 and 16, the periods critical for the development of sexual dimorphism. To assess for any developmental abnormalities, we measured the birth-weights, developmental weights, and litter sizes. Analysis showed no differences between groups, indicating normal development. However, we found an effect of treatment on the size of the prostate glands of male mice in adulthood, where testosterone treated mice exhibited smaller prostate weights. This demonstrates that our treatment was effective as early excess testosterone has been shown to down-regulate androgenic-feedback in the prostate. We then tested social motivation during preadolescence using the social approach/avoidance paradigm. Each mouse received a choice between a novel same-sex conspecific and an unfamiliar scent (vanilla). Results show that there was no significant effect of prenatal testosterone exposure in this test, suggesting that treatment did not affect motivation to approach social stimuli. Hence, the same dosage that was effective on peripheral tissues (prostate) did not affect a fundamental test of social motivation. Follow up investigations assessing other social behaviors such as social recognition and agonistic behavior will determine whether cognitively more complex social behaviors are influenced by the prenatal hormonal environment. We will also investigate interactions between the prenatal developmental and adulthood activational effects of the sex hormones on social behaviors. Our findings will help elucidate how hormones mediate sociability and will allow for better insight into developmental disorders, such as Autism Spectrum Disorder, where an abnormal social phenotype is apparent. Supported by the Ontario Mental Health Foundation.

Disclosures: **C.S. Wasson:** None. **C. Howes:** None. **M. Castro:** None. **A. Smart:** None. **A.J. Giuga:** None. **N.J. MacLusky:** None. **E. Choleris:** None.

Poster

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Topic: E.03. Behavioral Neuroendocrinology

Support: OMHF Grant 051449

Title: The effects of hyperandrogenic prenatal environment on later life social learning and gonadal hormone sensitivity

Authors: *C. HOWES, C. S. WASSON, M. CASTRO, A. SMART, A. J. GIUGA, N. J. MACLUSKY, E. CHOLERIS;
Univ. of Guelph, Guelph, ON, Canada

Abstract: Colin Howes, Cameron S Wasson, Anastasia Smart, Marian Castro, Anthony Giuga, Neil J MacLusky, and Elena Choleris Department of Psychology and Neuroscience Program Department of Biomedical Sciences University of Guelph Gonadal hormones and their downstream effectors are involved in the regulation of a number of social cognitive behaviours, such as social learning, social recognition, and social motivation in adult animals. In addition, testosterone has been shown to exert lasting developmental effects on sexual and affiliative behaviours. This indicates that the action of testosterone during development may be also be involved in the mediation of social cognition in later life. In the present study, we examined the developmental effects of testosterone on social learning in mice. Injections of 10 g testosterone propionate or sesame oil control were administered to pregnant CD1 mice on embryonic days 12, 14, and 16. This time period corresponds with critical periods for sexual differentiation and the development of socially relevant brain regions. Mice were tested on a number of social tasks prior to the onset of puberty. Social learning was assessed using the social transmission of food preferences task, in which experimental "observer" animals were allowed to interact with a "demonstrator" conspecific that had recently consumed one of two novel flavoured diets. Following this interaction, the observers were given access to both the demonstrated and non-demonstrated foods, and consumption was compared in order to discern whether or not they exhibited a socially acquired preference for the demonstrated food flavour. Preliminary data suggest that prenatal testosterone treatment had an impairing effect on social learning in male, but not in female mice. This suggests that testosterone may exert sexually dimorphic effects on social cognitive behaviour during development, which is in line with findings from human studies of autistic populations. Animals will undergo further testing to assess the effects of gonadal hormones in adulthood. In addition, we will correlate a number of physiological and brain measures with behavioural data in order to elucidate the possible mechanisms by which gonadal hormones act in development and later life to mediate social behaviour. This study will further illuminate how interplay between the developmental and activational effects of hormones produces a social behaviour phenotype. Supported by the Ontario Mental Health Foundation.

Disclosures: C. Howes: None. **C.S. Wasson:** None. **M. Castro:** None. **A. Smart:** None. **A.J. Giuga:** None. **N.J. MacLusky:** None. **E. Choleris:** None.

Poster

717. Social Behavior

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Topic: E.03. Behavioral Neuroendocrinology

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Title: Investigation of neural activity during social eavesdropping in male golden hamsters using c-Fos immunohistochemistry and local field potential recording

Authors: *C.-Y. LIU, W.-C. YU, C.-Y. CHANG, T.-R. HUANG, W.-S. LAI;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Social eavesdropping is a special type of social learning and it is defined as the act of extracting information about the relative or absolute quality of signalers from social interactions between conspecifics. Social eavesdropping has advantage in information gathering and has attracted increasing attention. However, this behavioral phenomenon and its underlying neural mechanisms remain much unclear. Taking advantage of agonistic behaviors in male golden hamsters and using the established social eavesdropping model in golden hamsters, we investigated brain areas and related neural activity underlying social eavesdropping in hamsters with a single defeated experience. Three groups of hamsters that were exposed to either a fighting interaction, a neutral encounter, or an empty arena were tested. In experiment 1, using c-Fos immunohistochemistry to map neuronal activities in the brain, males in the fighting interaction group had more c-Fos labeled neurons in the piriform cortex and sub-regions of cingulate cortex (especially anterior mid-cingulate cortex, aMCC) compared with males in the other two groups. But no significant difference was found in the hippocampus and amygdala. Based on the findings in experiment 1, local field potential was recorded in the aMCC of behaving hamsters to reveal neural activity during social eavesdropping in experiment 2. Neuronal tracing technique was further applied to identify neuronal pathways and projection areas of the aMCC in experiment 3. Our preliminary results reveal that the neurons of the aMCC seem to oscillate faster during fighting interactions compared with neutral interactions. Data collection and analyses are still in progress. Collectively, our data suggest that hamsters are capable of social eavesdropping and the aMCC might play an important role in the information gathering and extracting process during social eavesdropping.

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Poster

717. Social Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 717.05/Y5

Topic: F.03. Motivation and Emotion

Title: GABA neurons in the BNST and MA respond to social stimuli

Authors: *N. RIGNEY, K. MCDANIEL, A. PETRULIS;
Georgia State Univ., Atlanta, GA

Abstract: The medial amygdala (MA), bed nucleus of the stria terminalis (BNST) and the medial preoptic area (MPOA) are all critical brain regions for the regulation of social behavior. However, the chemical nature of socially responsive neurons in these areas is generally unknown. This study examines the co-localization of glutamic acid decarboxylase (GAD-67), the rate-limiting enzyme for GABA synthesis, with c-Fos gene activity (a marker for neuronal activation) in neurons of male mice following exposure to male and female stimuli. We use a mouse strain in which green fluorescent protein (GFP) is linked to an allele of the GAD-67 gene, thus allowing us to visualize GABAergic neurons, and c-Fos co-localization, across sub-regions of MA, BNST, and MPOA in response to male, female or control stimuli. However, we observed that these transgenic mice have both low levels of c-Fos expression and social deficits, likely due to decreases in GABA synthesis. GAD67-GFP mice investigated social stimuli less and produced less ultrasonic vocalizations than controls. Consequently, we are examining social behavior and c-Fos/GABAergic neuron co-localization in male offspring of vesicular GABA transporter (VGAT)-Cre mice crossed with lox-GFP reporter mice. Furthermore, using Cre-dependent viral-vector mediated delivery of inhibitory designer receptors exclusively activated by designer drugs (DREADD) to the BNST we are testing the necessity of GABAergic BNST neuron activity on production (ultrasonic vocalizations, urine marking) and reception (investigation, approach) of communication signals. These experiments provide critical information about the chemical identity of social-responsive neurons in the limbic brain and will greatly increase our understanding of social brain organization.

Disclosures: **N. Rigney:** None. **K. McDaniel:** None. **A. Petrulis:** None.

Poster

717. Social Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 717.06/Y6

Topic: E.03. Behavioral Neuroendocrinology

Support: NSRC 402417-2011

Title: Rapid formation of social memories in songbirds

Authors: *D. C. TOCCALINO[1], H. SUN[2], J. T. SAKATA[3];
[1]McGill University, IPN, Montreal, QC, Canada; [2]Neurosci., [3]Biol., McGill Univ., Montreal, QC, Canada

Abstract: Individual recognition is a form of social memory that shapes the expression of social behavior. To reveal behavioral indices of individual recognition and factors that influence the formation of social memories, we assessed how brief (<30 sec) and repeated exposures to females affected the expression of courtship behavior in male Bengalese finches, *Lonchura striata* var. *domestica* . Using a habituation-dishabituation paradigm, we analyzed the degree to which the prevalence and structure of courtship songs changed with repeated exposures to an individual female. Courtship behavior toward individual females rapidly changed following a single exposure. For example, males were significantly less likely to produce courtship song to an individual female upon their second exposure to that female. In addition, males produced progressively shorter songs to an individual female with repeated exposures. In contrast to song duration, other features of song that are affected by social interactions with females (e.g., stereotypy of syllable structure and sequencing, song tempo) did not change across repeated exposures to an individual female. Exposure to a different female increased the probability of courtship behavior and the duration of courtship songs. To assess the persistence of social memory, we also investigated the degree to which males differentially courted novel vs. familiar females two days after initial exposures. We observed that males preferentially courted novel females over females they were exposed to at least three times, suggesting that repeated exposures result in the formation of a persistent social memory. Single exposures, however, failed to induce the formation of persistent social memories for individual females. Taken together, these experiments demonstrate that interactions with females rapidly induce the formation of social memories and that multiple exposures are required for more persistent memory formation. In addition, these studies reveal that some features of courtship behavior are more sensitive indicators of social memory than others.

Disclosures: **D.C. Toccalino:** None. **H. Sun:** None. **J.T. Sakata:** None.

Poster

717. Social Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 717.07/Y7

Topic: F.03. Motivation and Emotion

Support: JSPS KAKENHI Grant Number 26118514

JSPS KAKENHI Grant Number 24500481.

Title: Rats demonstrate helping behavior toward a soaked cagemate

Authors: ***N. SATO**, L. TAN, K. TATE, M. OAKADA;
Kwansei Gakuin Univ., Nishinomiya, Hyogo, Japan

Abstract: Helping behavior is a prosocial behavior whereby an individual helps another irrespective of disadvantages to him or herself. To examine helping behavior in rats, we used a pool of water to create distress. We examined whether rats would help distressed, conspecific rats that had been soaked with water. To help a distressed cagemate, helper rats were required to open a circle-shaped door. The rats quickly learned to rescue a soaked cagemate from the water area by opening the door to allow the trapped rat into a safe area. We also examined the rats' preference regarding water and confirmed that the rats dislike soaking. Additional tests showed that the presentation of a distressed cagemate was necessary to induce rapid door-opening behavior. In addition, it was shown that rats that had previously experienced a soaking were quicker to learn how to help a cagemate than those that had never been soaked. The results using another rats indicated that they did not open the door to a cagemate that was not distressed. We also tested rats' behavior when they were forced to choose between opening the door to help a distressed cagemate and opening a different door to obtain a food reward. In half of the helper rats, the door-opening behavior was shaped by helping the cagemate. In the remaining half of rats, the door-opening behavior was shaped by food reward. Irrespective of how they learned to open the door, in most test trials, rats chose to help the cagemate before obtaining a food reward, suggesting that the relative value of helping others is greater than the value of a food reward. These results suggest that rats can behave prosocially and that helper rats may be motivated by empathy-like feelings towards their distressed cagemate. The paradigm in the present study can be a useful tool for understanding the underlying neural basis of prosocial behavior.

Disclosures: **N. Sato:** None. **L. Tan:** None. **K. Tate:** None. **M. Oakada:** None.

Poster

717. Social Behavior

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 717.08/Y8

Topic: E.03. Behavioral Neuroendocrinology

Support: Portland VA Research Foundation

Title: Early post-natal sleep fragmentation prevents normal social development in male, but not female, prairie voles

Authors: E. A. D. HAMMOCK[1], D. L. COCKING[2], *M. M. LIM[2];
[1]Psychology, Florida State Univ., Tallahassee, FL; [2]Sleep Med., Portland VA / Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Consolidated sleep periods during development are critical for synaptic plasticity and pruning in basic brain circuits (such as the visual system). However, it is unknown whether sleep plays a role in the maturation of more complex circuits, such as those involved in species-typical social behavior. Prairie voles (Microtus ochrogaster) are a highly social rodent species and form lifelong pair bonds with other individuals, thus providing an ideal model organism to study the role of sleep in shaping social function. We sought to establish an animal model to directly investigate the effects of sleep on the development of neural systems involved in social behavior. We applied a unique method of chronic sleep fragmentation, which preserves total sleep times and is relatively stress-free, to prairie vole pups during a sensitive post-natal period of development. Following developmental sleep fragmentation, voles underwent tests for social behavior as juveniles, and again as adults. At 5 weeks of age, juvenile male prairie voles showed increased aggression towards age-matched stranger voles, regardless of sleep intervention. At 11 weeks of age, adult male prairie voles subjected to sleep fragmentation in the post-natal period showed persistence of aggressive behavior towards juvenile voles, in contrast to non-sleep-fragmented male prairie voles. Furthermore, adult male prairie voles sleep-fragmented during the post-natal period showed profoundly impaired pair bond formation as assessed by the partner preference test. Sleep fragmentation during post-natal development did not significantly affect olfactory memory function, nor did it affect social behavior in female prairie voles. This sex-specific effect of sleep fragmentation on partner preference is reminiscent of human disorders of social behavior showing a male-biased prevalence, including autism spectrum disorders. Our data suggest that sleep fragmentation during the pre-weaning post-natal period may halt the

normal developmental trajectory of social behaviors, including persistence of aggression and an inability to form pair bonds. Future studies will examine markers of synaptic plasticity and neuronal activity, oxytocin receptors, vasopressin receptors, and GABA interneurons. Studies utilizing this unique animal model will enhance our understanding of modifiable risk factors, such as sleep, that may contribute to atypical development of the brain and social behavior.

Disclosures: E.A.D. Hammock: None. **D.L. Cocking:** None. **M.M. Lim:** None.

Poster

717. Social Behavior

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Program#/Poster#: 717.09/Y9

Topic: E.03. Behavioral Neuroendocrinology

Support: Pilot Award Center for Complicated Grief, Columbia University

Collaborative and Multidisciplinary Pilot Research Award, Columbia University

Title: Neural architecture of a pair bond: calcium imaging of the nucleus accumbens in awake-behaving prairie voles

Authors: E. CARAZO[1], A. M. CUNNINGHAM[1], R. HEN[1,2], M. A. KHEIRBEK[1,2], *Z. R. DONALDSON[1,2];
[1]Columbia Univ., New York, NY; [2]New York State Psychiatric Inst., New York, NY

Abstract: Despite the strong evidence that social bonds impact numerous aspects of human health, the neural basis for bonding remains poorly understood. This is in part due to the relative shortage of appropriate animal models; biomedical research relies heavily on rats and mice, but these animals do not form strong, selective bonds with other adult conspecifics. However, laboratory-amenable prairie voles offer further opportunities to understand how social bonds are formed and maintained because, as a monogamous species, they form intense bonds between mated partners. Multiple lines of converging evidence suggest that the nucleus accumbens (NAcc) plays a critical role in pair bonding. Monogamous prairie voles exhibit high levels of oxytocin receptors in the NAcc. Local antagonists or reduction of oxytocin receptors in the NAcc inhibits bond formation, while increasing levels of these receptors can enhance bond formation. In addition, both dopamine D2 and μ-opioid receptor signaling within the shell of the NAcc are required for bonding. Further, plasticity in gene expression within this brain region has been observed following pair bond formation. This suggests that coordinated action of multiple

neuromodulatory systems converge in the NAcc during pair bond formation and that neuroplastic changes within this region may help to maintain social bonds. However, despite the clear importance of the NAcc in pair bonding, very little is known about the local circuit within the NAcc that mediates this behavior, and how this circuit changes upon bond formation and over time. In order to understand the neural circuits that underlie the formation of social bonds, we have used calcium imaging *in vivo* to monitor activity of large populations of NAcc neurons in prairie voles during epochs of social interaction. This allowed us to observe population activity in response to different social and non-social stimuli before and after bond formation. Analysis of NAcc neuron firing rate reveal that these neurons code for both the type of stimulus (social versus non-social) and the type of the social interaction. Our ongoing efforts are aimed at identifying how this population code within the NAcc is related to interactions with a bonded partner versus a novel conspecific and how this changes with time after bonding.

Disclosures: **E. Carazo:** None. **A.M. Cunningham:** None. **R. Hen:** None. **M.A. Kheirbek:** None. **Z.R. Donaldson:** None.

Poster

717. Social Behavior

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Program#/Poster#: 717.10/Y10

Topic: F.04. Neuroethology

Support: NSF CAREER Award 1149446

Title: Longitudinal analysis of individual, social and prosocial behaviors in a naked mole-rat colony

Authors: M. SANSONE[1], M. STENDARDI[2], L. OVEREEM[2], T. DZEDZITS[5], M. KRESS[3], E. MEEHAN[2], *D. P. MCCLOSKEY[4,5];
[1]Master's Program in Neurosci. & Developmental Disabilities, [2]Dept. of Psychology, [3]Vice President for Information Technol., [4]Dept of Psychology and Program in Developmental Neurosci., City Univ. of New York, Staten Island, NY; [5]PhD Program in Psychology, Grad. Ctr. at CUNY, New York, NY

Abstract: The African Naked Mole-Rat (H. Glaber), a fossorial rodent, is one of only two mammalian species identified to have a eusocial organization and cooperative breeding system. This unique social hierarchy, along with the extreme longevity of this species, allows for the measurement of individual and group behaviors under laboratory conditions, in large groups (>

30 animals) over a long time span (> 1 year). This study analyzed the stability of individual and group measures within a single colony at two or more timepoints over a 1 year period. Measures included individual characteristics (weight, sex, cortisol levels, hierarchy position, activity, and diurnal rhythm), social behaviors (animal proximity) and prosocial colony behaviors (nest-building, pup-carrying, tunnel excavating, and threat response). Measurement of these behaviors involved Radio Frequency Identification (RFID) and computational approaches to complement observational data. Results supported a "division of labor" model of task performance, and demonstrated a significant correlation over time for the contribution of each animal to nest building and tunnel excavating, suggesting stability of these roles. Measures of pup-carrying and animal proximity (degree centrality of colony social network) were less stable. The demonstrated stability of some colony roles will allow for measures of epigenetic factors that determine social hierarchy. -The first two authors contributed equally to this work.

Disclosures: **M. Sansone:** Other; Contributed equally to this work. **M. Stendardi:** Other; Contributed equally to this work. **L. Overeem:** None. **T. Dzedzits:** None. **M. Kress:** None. **E. Meehan:** None. **D.P. McCloskey:** None.

Poster

718. Social Behavior: Genetic and Molecular Basis

Location: Hall A

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Topic: F.03. Motivation and Emotion

Support: NIH Grant MH080759

Autism Speaks Translational Postdoctoral Fellowship 7595

Title: Understanding heterogeneity in social behavior using QTL mapping in BXD mouse strains

Authors: *A. T. KNOLL[1], N. FOX[2], P. LEVITT[1];
[1]Inst. for the Developing Mind, Children's Hosp. Los Angeles, Los Angeles, CA; [2]Dept. of Human Develop., Univ. of Maryland, College Park, MD

Abstract: Humans exhibit broad heterogeneity in affiliative social behavior. Twin and family studies demonstrate that individual differences in core dimensions of social behavior are heritable, yet there are knowledge gaps in understanding the underlying genetic and neurobiological mechanisms. In neurodevelopmental disorders, there is also remarkable heterogeneity in social dysfunction, even in individuals with the same causal mutation, and this

often negatively impacts the efficacy of clinical treatments. We hypothesize that the genes that cause variation in typical social behavior act in concert with disorder-related genes to influence clinical heterogeneity. Here, we report initial studies using the BXD genetic reference panel of mice to address the heritable nature of heterogeneity in social behaviors. The BXD panel is comprised of >100 recombinant inbred strains derived from the C57BL/6 (B6) and DBA/2 (D2) parental strains. The B6 and D2 parental strains show high levels of sequence variation (~2 million informative single nucleotide polymorphisms (SNPs)), and all BXD strains have been sequenced at >7000 informative SNPs, enabling rapid quantitative trait locus (QTL) mapping. We examined four domains of affiliative social behavior: social approach motivation, social recognition, direct social interaction motivation, and communication. Using a large subset of BXD strains, multiple measures were obtained, including data collected from the 3-chamber social interaction task and a direct social interaction (DSI) task with synchronous ultrasonic vocalization (USV) recording. There was a several hundred-fold range in quantitative traits, with moderate to high heritability (h2) of USV count (h2 = 0.33) and the percentage of time spent sniffing a social partner in DSI (h2 = 0.50). Continuous measures collected for each task were used for QTL mapping, with several novel QTLs identified for social approach, direct social interaction, and USV communication. Bioinformatics tools were used to identify functional polymorphisms within candidate quantitative trait genes (QTGs), and brain regions and biological networks in which they might function to influence social behavior, resulting in a prioritized list of 10 candidate QTGs. These data will guide next step opportunities for determining gene by environment factors that influence social behavior heterogeneity, which is essential for understanding typical social development and addressing disorder risk in children and families.

Disclosures: **A.T. Knoll:** None. **N. Fox:** None. **P. Levitt:** None.

Poster

718. Social Behavior: Genetic and Molecular Basis

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Topic: F.03. Motivation and Emotion

Support: NSF IIS 1029373

NIH MH080759

Autism Speaks Translational Postdoctoral Fellowship 7595

Title: MUPET - A novel software tool for high-throughput analysis of mouse ultrasonic vocalizations

Authors: M. VAN SEGBROECK[1], A. T. KNOLL[2], *P. R. LEVITT[2], S. NARAYANAN[1]; [1]Dept. of Electrical Engin., USC, Los Angeles, CA; [2]Children's Hosp. Los Angeles, Los Angeles, CA

Abstract: Social communication across species includes the use of different forms of vocalizations. Analysis in animal models typically has utilized a limited number of spectro-temporal measures to compare vocalizations generated in different environmental contexts or following genetic manipulation. A few in depth analyses have revealed a level of communication complexity that will likely require new analytical tools that enable higher throughput processing of hundreds of hours of vocalizations. Measures must also be sufficiently sensitive to detect subtle variations in vocal production and syllable repertoires. Here, we report the development of MUPET (Mouse Ultrasonic Profile ExTraction) software, an open access MATLAB® tool with graphical user interface that provides a data-driven, high-throughput analysis of mouse ultrasonic vocalizations (USVs). Using a fully automated and unsupervised algorithmic approach, MUPET has five core capabilities that enable it to detect, learn, and compare syllable patterns and repertoires: 1) syllable detection--the isolation and measurement of basic spectro-temporal call parameters, 2) acoustical dataset analysis--statistical analysis of overall vocalization features, such as number of syllables, syllable rate, syllable duration, and frequency bandwidth, 3) repertoire learning--the extraction of up to several hundred of the most highly represented syllable patterns ("repertoire units") in individual datasets, 4) syllable category counts--a categorical cluster analysis of syllable-like patterns across different dataset repertoires to measure the frequency of use of different syllable types, and 5) measures of repertoire similarity--rank order comparisons of the similarity of spectral shapes of individual repertoire units across datasets. MUPET allows the user to rapidly analyze thousands of calls, facilitating the comparison of fine details of vocal production and repertoire use across large numbers of mouse lines and experimental conditions. Proof of principle was accomplished using USV data collected from mice in the BXD genetic reference panel, which we have shown exhibits wide-ranging social communication competencies. MUPET analysis revealed genetic control of USV counts, vocal production and repertoire sampling, which are at least partially independent of the social context. MUPET is a powerful new tool for the automated analysis of mouse USVs and has the flexibility to be applied to USV datasets collected from different species. MUPET is also open to the scientific community for the incorporation of additional analytical features.

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Poster

718. Social Behavior: Genetic and Molecular Basis

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Program#/Poster#: 718.03/Y13

Topic: F.03. Motivation and Emotion

Support: KAKENHI (23683021)

Title: Mapping of genetic factors for intermale aggressive behavior on mouse chromosome 15

Authors: *A. TAKAHASHI[1,2], H. SUGIMOTO[4], S. KATO[5], T. SHIROISHI[3], T. KOIDE[2]; [1]Univ. of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Mouse Genomics Resource Lab., [3]Mammalian Genet. Lab., Natl. Inst. of Genet., Mishima, Japan; [4]Ctr. for Mol. Med., Jichi Med. Univ., Shimotsuke, Japan; [5]The Inst. of Statistical Mathematics, Tachikawa, Japan

Abstract: Aggression has large individual difference, as well as wide-range of conservation among animal kingdom. Despite high level of heritability estimates for aggressive trait, genetic basis for individual difference of aggression is still unclear. Japanese wild-derived mouse strain MSM/Ms (MSM) has intensively high level of aggressive behaviors compared to standard laboratory strain C57BL/6J (B6). Almost all males of MSM showed high frequency of attack bites and pursuit in the resident-intruder test, while only a few males of B6 showed aggressive behavior with low frequency. In addition, MSM showed killing behavior of his littermates, or sometimes female pair-mates, in his home cage after the sexual maturation. We identified that chromosome 15 (Chr 15) has one of the genetic factors of escalated aggression observed in MSM by using a panel of consomic strains of MSM on B6 background. To narrow down the genetic loci involved in the enhancement of aggressive behavior on Chr 15, we established a panel of subconsomic strains of MSM Chr 15. Analysis of the subconsomic strains suggested the existence of multiple genes that enhance and suppress aggressive behavior even within Chr 15, and those loci have complex interactions. The least squares linear regression analysis successfully identified four genetic loci involved in aggressive behavior on the Chr 15, and one of the genetic loci, which partially enhances aggressive behaviors, was narrowed down into a 4.1 Mbp region of the Chr 15.

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Poster

718. Social Behavior: Genetic and Molecular Basis

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH grants for Dr.s Fernald and Snyder

Title: Differential methylation as a function of social status in a cichlid fish, Astatotilapia burtoni

Authors: *A. T. HILLIARD[1], D. XIE[2], Z. MA[2], M. SNYDER[2], R. D. FERNALD[1];
[1]Dept. of Biol., [2]Dept. of Genet., Stanford Univ., Stanford, CA

Abstract: Social status is an important feature of all social systems and the effects of low status have well-known negative health consequences. However, the molecular systems underlying social behavior are complex and not well understood. While some specific genes have been linked to social behavior (e.g. hormone receptors), the nature of their regulation in social contexts remains unclear. DNA methylation is a promising candidate regulatory mechanism, as it has recently been linked to social behavior in multiple species. In addition, unpublished work in our lab has shown that methylation can influence social rank in Astatotilapia burtoni, a cichlid fish with a dynamic social system based on male status. Dominant (D) A. burtoni males defend territories, are reproductively capable, display bright colors, and perform a number of aggressive and courtship behaviors. In contrast, non-dominant (ND) males cannot establish territories or reproduce, are drably colored, and display a limited behavioral repertoire. Here, our aim was to broadly characterize the molecular pathways and functions that are differentially affected by DNA methylation in the brains of D and ND males. To ensure that our samples were representative of distinct D and ND physiological states, we placed size-matched males in dyads where a stable D-ND relationship was established and maintained for weeks. Since multiple hypothalamic sub-regions are members of a conserved set of neural circuits that regulate vertebrate social behavior, we collected hypothalamus tissue from two D and two ND males in stable D-ND dyads. To establish status-related methylation patterns in an unbiased manner we isolated DNA and performed whole-genome bisulfite sequencing, a method that allows quantification of methylation levels at single base-pair resolution across the entire genome. To assess whether potential methylation differences affected gene expression we also sequenced RNA from the same samples. We used the BSmooth algorithm to generate smoothed methylation profiles for each fish and identified genomic regions that were differentially methylated in D vs ND fish (DMRs). Some of the most significant DMRs were directly upstream of, or within, genes differentially expressed in a manner consistent with the DMR, including genes involved in transcriptional regulation, protein degradation and trafficking, cell growth, and calcium ion activity. Deeper analysis of these results will allow us to optimize the design of future necessity/sufficiency studies by highlighting the most promising candidate genes and pathways,

especially when combined with findings from other large-scale gene expression studies in our lab.

Disclosures: **A.T. Hilliard:** None. **D. Xie:** None. **Z. Ma:** None. **M. Snyder:** None. **R.D. Fernald:** None.

Poster

718. Social Behavior: Genetic and Molecular Basis

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Topic: E.03. Behavioral Neuroendocrinology

Support: PTDC/MAR/69749/2006

EXCL/BIA-ANM/0549/2012

012/2012/A1

Title: Neurogenomics of alternative reproductive tactics in the blenniid fish Salaria pavo

Authors: ***S. D. CARDOSO**[1,2], D. GONÇALVES[3], A. GOESMANN[4], A. V. M. CANÁRIO[5], R. F. OLIVEIRA[1,2];
[1]Inst. Gulbenkian De Ciência, Oeiras, Portugal; [2]Champalimaud Neurosci. Programme, Champalimaud Ctr. for the Unknown, Lisbon, Portugal; [3]Inst. of Sci. and Environment, Univ. of St. Joseph, Macau SAR, China; [4]Bioinformatics and Systems Biol., Justus-Liebig-University, Giessen, Germany; [5]CCMAR – Ctr. de Ciências do Mar, Univ. do Algarve, Faro, Portugal

Abstract: Organisms that share the same genotype can develop into different phenotypes depending on perceived environmental condition cues (aka phenotypic plasticity). Although not without its costs, phenotypic plasticity has been considered to facilitate adaptive evolution by allowing organisms to exploit a wider spectrum of resources when their fitness is reduced. Particularly in species with discrete variation in reproductive behavior, different life-history trajectories are observed that can either be fixed (e.g. genetic polymorphism), or plastic and therefore responsive to environmental factors (e.g. time of birth, resource availability) that will ultimately influence developmental processes. In the peacock blenny Salaria pavo males can follow either of two developmental pathways, grow directly into dominant nest-holder males or mimic the females' morphology and courtship displays in order to approach the nests of larger males to sneak fertilizations. These alternative reproductive tactics (ARTs) are sequential and consistent with body size (i.e. size at sexual maturity), such that sneaker males in the follow

breeding season switch to nest-holder males, going through a phase in which they are reproductively inactive (i.e. transitional males). In this work, we explored at the neurogenomic level how gene expression profiles differed between male morphs (i.e. nest-holder, sneaker and transitional males) and females. For this, RNA was isolated from whole brain tissue for each one of the four phenotypes, sequenced using Illumina HiSeq 2000 and de novo assembled. We found that at the brain level, expression of the plastic male tactic was accompanied by broader and divergent gene expression when compared to either females or nest-holder-males, both more similar between themselves. Overall, sneaker males differed from the other phenotypes in the expression of 643 unique transcripts (78.38% annotated), followed by transitional males (597 transcripts; 75.71% annotated), females (562 transcripts; 76.51% annotated) and finally nest-holder males (517 transcripts; 78.92% annotated). Given the unique dichotomy present in sneaker males of the peacock blenny, behaviorally female-mimics but mature reproductive males, the lack of convergence between brain gene expression and behavior in sneakers to either females or nest-holders points to a differential modulation of underlying proximate mechanisms of sexual behavior in sneakers.

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Poster

718. Social Behavior: Genetic and Molecular Basis

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Program#/Poster#: 718.06/Y16

Topic: F.04. Neuroethology

Support: NIGMS 101095

NINDS 034950

Stanford Bio-X Bowes Fellowship

Title: The neurogenomic substrate of and extended phenotype in Lake Malawi cichlid fish

Authors: *R. YORK[1], H. FRASER[2], R. D. FERNALD[2], J. T. STREELMAN[3];
[1]Biol., Stanford Univ., LA Honda, CA; [2]Biol., Stanford Univ., Stanford, CA; [3]Georgia Inst. of Technol., Atlanta, GA

Abstract: Identifying the proximate and ultimate causes behind behavioral evolution is one of biology's central challenges. To do so requires integrating methods from multiple disciplines in

order to analyze animal clades that show substantial variation in tractable behaviors. To this end we studied two species from the bower (mating nest) building clade of Lake Malawi cichlid fish: Copadichromis virginalis ("CV"; pit-digger) and Mchenga conophoros ("MC"; castle-builder). We tested for cis-regulatory divergence between CV and MC by analyzing allele-specific expression (ASE) in whole brain RNA-seq data from CVxMC F1 hybrids. We identified over 150 genes with significant allele-specific regulatory changes driven by bower-building through the comparison of differential ASE in behaving and isolated F1 hybrids. We found evidence for lineage-specific selection at the expression level for several sets of genes in known pathways involved in neurophysiological processes such as ion transport. Because our RNA-seq results lack information about the spatial context in which these genes are functioning we next performed *in situ* hybridization targeting immediate early genes (IEGs) indicative of recent neural activity. These experiments identified several neural loci associated with bower building including the vagal lobe of the dorsal medulla. Finally we compared the brain expression patterns of candidate genes from the differential ASE analysis with these behaviorally associated brain regions. Our results suggest that the cis-regulatory divergence of genes involved in adult neural function may play an important role in the evolution of cichlid fish behavior.

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Poster

718. Social Behavior: Genetic and Molecular Basis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 718.07/Y17

Topic: F.03. Motivation and Emotion

Title: Creatine transporter knockout mice show increased anxiety, increased depressive-like behaviors, and reductions in sociability

Authors: *A. N. KOKENGE[1,2], M. R. SKELTON[1,2];
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Abstract: Creatine deficiency syndromes are a family of disorders characterized by intellectual disability, aphasia, and epilepsy. The most common cause of Cr deficiency is Creatine Transporter Deficiency (CTD) which is the second leading cause of X-linked intellectual disability (XLID). We have developed a high-fidelity model for CTD, the creatine transporter (CrT) knockout ($CrT^{-/y}$) mouse. While the primary goal of the work to date has focused on the cognitive deficits, it is important to evaluate affective behaviors in these animals. Cr has been implicated in depressive-like behaviors in the mouse and rat; therefore, we set out to determine if

$CrT^{-/y}$ mice showed changes in anxiety and depressive-like behaviors. $CrT^{-/y}$ mice appeared to be more anxious than $CrT^{+/y}$ mice, spending less time in the open arms and committing fewer head dips in the elevated zero maze compared to $CrT^{+/y}$ mice. When tested in the tail-suspension test, $CrT^{-/y}$ mice had a decreased latency to immobility compared with $CrT^{+/y}$ mice, suggesting moderate depression-like behavior. Finally, sociability was reduced in the $CrT^{-/y}$ mice with $CrT^{-/y}$ mice spending less time with the novel stranger compared with $CrT^{+/y}$. These results suggest that Cr plays an important role in affective behaviors. Further, these results suggest that patients with Cr deficiencies need to be monitored for changes in these behaviors during treatment.

Disclosures: A.N. Kokenge: None. **M.R. Skelton:** None.

Poster

718. Social Behavior: Genetic and Molecular Basis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 718.08/Y18

Topic: F.03. Motivation and Emotion

Support: NIH Grant 1R01MH105447-01

Title: Altered DNA methylation pattern in the amygdala of rats genetically prone to high versus low anxiety

Authors: *C. R. MCCOY, N. L. JACKSON, T. PTACEK, E. J. LEFKOWITZ, J. J. DAY, S. M. CLINTON;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Individual differences in emotionality contribute to vulnerability for mood disorders such as depression and anxiety. Understanding molecular mechanisms that drive these temperamental differences is crucial for generating improved treatments. To study neurobiological factors that influence emotionality, we use model rats that were bred based on a high versus low behavioral response to novelty. Rats bred for low novelty response (bLR) exhibit a high anxiety-/depressive-like phenotype compared to high novelty responder rats (bHRs), which vigorously explore novelty and exhibit high impulsivity, aggression, and risk taking. We previously found dramatic bHR/bLR gene expression differences and global DNA methylation differences in the early postnatal and adult amygdala. These data led us to hypothesize that individual differences in neural DNA methylation patterns contribute to the disparate bHR/bLR phenotypes. To obtain a more detailed understanding of DNA methylation differences in the adult bHR/bLR amygdala, the current study used next-generation sequencing

to map the methylation landscapes of adult bHR/bLR amygdala. Methylated DNA was immunoprecipitated and prepared for high-throughput sequencing on Illumina HiSeq2000. We examined DNA methylation at CpG sites using MEDIPS, a R Bioconductor program that compared methylation patterns across CpG islands, intragenic and other regulatory regions in bHR/bLR samples. We cross-referenced our methyome sequencing results with prior bHR/bLR amygdala transcriptome data to identify genes that were differentially expressed and also showed methylation differences in the gene promoter or gene body. To examine functional implications of bHR/bLR DNA methylation differences, another study manipulated methylation levels in bLR rats through diet, feeding them either a methyl donor-depleted, methyl donor-supplemented, or control diet for 4 weeks. Rats then embarked on a behavioral test battery that included the Elevated Plus Maze (EPM), Open Field test, and Forced Swim Test (FST). Methyl donor supplementation improved bLRs' anxiety- and depression-like behavior, leading to greater EPM open arm exploration and a trend for diminished FST immobility. Methyl donor depletion, on the other hand, exacerbated bLRs' depression-like behavior, with methyl-depleted bLRs showing greater FST immobility than bLR controls. Overall these data suggest that inborn DNA methylation differences contribute to disparate bHR/bLR behavioral phenotypes and point to novel therapeutic strategies for individuals that exhibit high levels of anxiety- and depression-like behavior.

Disclosures: C.R. McCoy: None. **N.L. Jackson:** None. **T. Ptacek:** None. **E.J. Lefkowitz:** None. **J.J. Day:** None. **S.M. Clinton:** None.

Poster

718. Social Behavior: Genetic and Molecular Basis

Location: Hall A

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Program#/Poster#: 718.09/Y19

Topic: F.03. Motivation and Emotion

Support: NIH Grant 1R01MH105447-01

NIH Grant 5T32GM008111-27

Title: Early-life exposure to the SSRI paroxetine disrupts DNA methylation in the early postnatal hippocampus

Authors: *M. E. GLOVER, C. R. MCCOY, N. L. JACKSON, S. M. CLINTON;
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Abstract: Selective serotonin reuptake inhibitors (SSRIs) are the most common pharmacological treatment for major depression in pregnancy. The long-term effects of early-life SSRI exposure on human emotional health are largely unknown owing to insufficient study; however, in rodents, perinatal SSRI exposure can cause life-long adverse outcomes, elevating adult anxiety- and depression-like behavior. Furthermore, certain individuals appear to be more susceptible to adverse effects of perinatal SSRI exposure, but the mechanisms driving this differential vulnerability are completely unknown. We recently showed that rats selectively bred for low behavioral response to novelty (bLRs), which also exhibit high levels of spontaneous anxiety- and depression-like behavior, are vulnerable to the adverse effects of perinatal SSRIs. Perinatal SSRI exposure exacerbated bLRs' already high levels of depression-like behavior (Forced Swim Test (FST) immobility), while High Novelty Responder rats (bHRs) were unaffected by the treatment. We also conducted a transcriptome study that revealed widespread gene expression alterations in the early postnatal hippocampus (HPC) of perinatal SSRI-exposed bLR offspring, with striking SSRI-induced down regulation of several DNA methylation-related genes. Our new experiments tested the working hypothesis that the adverse behavioral consequences of perinatal SSRI exposure are mediated by perturbed DNA methylation in the early postnatal HPC. Our first study examined global DNA methylation (5-methylcytosine) levels in the postnatal day 15 HPC of bHR/bLR pups whose mothers were being treated with the SSRI paroxetine (10 mg/kg/day in drinking water) or vehicle (n=6/condition). Perinatal SSRI exposure led to decreased hippocampal DNA methylation levels specifically in bLR offspring relative to vehicle-exposed bLR controls; notably no changes were observed in other limbic brain regions. Interestingly, bHR offspring were not affected by SSRI exposure, with both SSRI- and vehicle-exposed bHR groups showing similar DNA methylation levels. Next generation sequencing analyses are currently underway to assess perinatal SSRI-induced gene-specific methylation changes in bLR offspring. Since we hypothesize that perinatal SSRI-induced suppression of DNA methylation leads to long-term behavioral effects such as increased FST immobility, our second study aims to suppress DNA methylation in early postnatal bLR rats using a methyl donor-depleted diet and then examining their adult behavior. The results of these studies will identify novel mechanisms in the etiology of depressive traits in a rodent model of SSRI susceptibility.

Disclosures: **M.E. Glover:** None. **C.R. McCoy:** None. **N.L. Jackson:** None. **S.M. Clinton:** None.

Poster

718. Social Behavior: Genetic and Molecular Basis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 718.10/Y20

Topic: F.03. Motivation and Emotion

Support: NIH Grant R00MH081927

Title: A2 noradrenergic neurons regulate forced swim test immobility

Authors: *H. NAM, I. KERMAN;
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Abstract: The Wistar-Kyoto rat (WKY) is a well-established animal model of depression- and anxiety-like behavior, characterized by high immobility during the forced swim test (FST) along with a generally inhibited phenotype on related tests of emotional behaviors. Extensive literature indicates that deficits in noradrenergic neurotransmission may contribute to these behavioral traits. Previously, we have reported that the WKY rats are more immobile compared to other rat strains from the beginning of their training phase of the FST, and that they become even more immobile during the testing phase on the next day. We hypothesized that higher immobility during the FST and the greater increase in immobility throughout different phases of the FST are two separate components of rats' behavior likely mediated by different central mechanisms. We sought to identify the central circuits responsible for these behavioral components by studying activation of neurons within central noradrenergic cell groups during different phases of the FST. The WKY rats along with its parent strain, Wistar rats that experienced either the: 1) 5 minutes training phase (D1), or 2) entire FST (D1 and D2) were compared. Using double-immunocytochemistry for tyrosine hydroxylase and for c-Fos, we determined that within the A2 cell group significantly more noradrenergic neurons were activated in the Wistar than in WKY rats at D1. At D2 WKYs increased their activation of the A2 noradrenergic neurons, and this activation was equivalent to that of the Wistar group. Based on these results, we further investigated the role of A2 cell group during the FST using anti-DBH conjugated saporin (DSAP) to selectively destroy noradrenergic neurons within the area. The Wistar rats treated with DSAP were more immobile during both D1 and D2 of the FST as compared to the rats treated with the vehicle only. Together these data indicate that the A2 noradrenergic cell group regulates FST immobility in rats, and that its activation may contribute to the unique behavioral phenotype of WKY rats. Future experiments aimed at selective activation of A2 noradrenergic neurons will be required to fully elucidate the role of these neurons in mediating behavioral despair and learned helplessness.

Disclosures: H. Nam: None. **I. Kerman:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 719.01/Y21

Topic: F.01. Human Cognition and Behavior

Support: DoD Contract: N66001-14-2-4032

Title: Multivariate analysis of electrical stimulation to predict memory performance

Authors: ***Y. EZZYAT**[1], J. BURKE[1], D. LEVY[1], A. LYALENKO[1], M. SPERLING[3], A. SHARAN[4], G. WORRELL[5], M. KUCEWICZ[5], B. JOBST[6], K. DAVIS[7], T. LUCAS[8], R. GROSS[10], B. LEGA[11], J. STEIN[9], S. DAS[2], D. RIZZUTO[1], M. KAHANA[1];
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Abstract: Memory function often declines with age and is disrupted in a variety of neurological disorders, but effective interventions are limited. Here we used targeted electrical stimulation of the human medial temporal lobe in an attempt to influence memory function in patients with epilepsy. In an initial passive recording session, patients undergoing intracranial monitoring performed a verbal free recall memory task while we recorded electrical activity from depth and cortical surface electrodes. We used the spectral decomposition of the electrode recordings to train a logistic regression classification model to discriminate brain-wide activity associated with successful and unsuccessful memory encoding. Within each patient, we analyzed recall performance according to the classifier's estimate of the probability that each trial would later be remembered. Recall performance was significantly higher in the top classifier quartile, compared to the bottom classifier quartile, validating the classifier's ability to discriminate remembered from forgotten trials. In a subsequent session, we then applied bipolar electrical stimulation while patients again performed the free recall task. We used the previously trained classifier model to estimate the probability that neural activity recorded after stimulation events was reflective of successful vs. unsuccessful memory encoding. Behaviorally, stimulation tended to impair memory performance on average, with memory improvements in some patients and impairments in others. Importantly, the behavioral effect of stimulation was predicted by the electrophysiological effects of stimulation, as estimated by the trained classifier's prediction of the probability of recall. These data demonstrate that machine learning classification can be used to decode neural activity to predict later memory, and that such decoding can be used to predict the behavioral effects of electrical stimulation. The findings suggest that such a process could be used to tune the parameters of stimulation or other neurological interventions intended to ameliorate memory dysfunction.

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Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 719.02/Y22

Topic: F.01. Human Cognition and Behavior

Title: Human hippocampal theta and its relationship to speed, memory, and voluntary movement

Authors: *J. MILLER[1], M. SPERLING[2], A. SHARAN[2], K. DAVIS[3], J. JACOBS[4]; [1]Drexel Univ., Philadelphia, PA; [2]Thomas Jefferson Univ. Hosp., Philadelphia, PA; [3]Hosp. of the Univ. of Pennsylvania, Philadelphia, PA; [4]Columbia Univ., New York, NY

Abstract: In rodents, the power and frequency of the hippocampal theta rhythm (4-8 Hz) is strongly modulated by ongoing behavior, particularly running speed. In contrast, human hippocampal theta is less prominent, and its relationship to movement is not well characterized. Electrocorticographic (ECoG) recordings from human hippocampus have shown increases in low frequency power associated with periods of virtual movement compared to periods of stillness, yet these previous tasks were not designed to explicitly examine the effects of movement speed. We designed a virtual spatial navigation and memory task well suited to elicit low frequency activity while systematically modulating speed of movement. Using data from neurosurgical patients with hippocampal depth electrodes, we provide evidence that movement related theta oscillations in human hippocampus reliably appear at lower (between 1-4 Hz) frequencies than in rodents and increase in power as movement speed increases. In addition, we use multivariate statistics to untangle the relative contributions of movement speed, memory performance, and voluntary vs involuntary movement to the overall spectral signal.

Disclosures: **J. Miller:** None. **M. Sperling:** None. **A. Sharan:** None. **K. Davis:** None. **J. Jacobs:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 719.03/Y23

Topic: F.01. Human Cognition and Behavior

Support: DARPA RAM (Restore Active Memories) N66001-14-2-4-31

Title: Dissecting induced high frequency activity in humans during short-term memory tasks and stimulation

Authors: *M. T. KUCEWICZ[1], B. M. BERRY[1], B. H. BRINKMANN[1], M. R. SPERLING[2], B. C. JOBST[3], R. E. GROSS[4], B. LEGA[5], J. M. STEIN[6], S. DAS[6], S. M. STEAD[1], D. S. RIZZUTO[7], M. J. KAHANA[7], G. A. WORRELL[1];
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Abstract: Intracranial recordings in humans have shown that induced high frequency power (30-150Hz) can be used as biomarker for mapping and stimulation of memory circuits (Johnson and Knight 2015, Curr Opin Neurobiol 31), but their physiological origins and function in the mechanisms of cognition remain elusive. Although animal studies provide evidence for synchronous oscillations generated by coordinated firing of neuronal assemblies, broadband asynchronous neural activities were also proposed to underpin high frequency power changes in human intracranial recordings (Burke et al. 2015, Curr Opin Neurobiol 31). Here we used a large dataset of intracranial recordings and stimulation in epilepsy patients (DARPA 'Restore Active Memories' project) to investigate the relative contribution of brain oscillations and asynchronous power increases to high frequency responses induced in two verbal short-term memory tasks. High frequency power changes were detected on a trial-by-trial basis and classified into low gamma (30-60 Hz), high gamma (60-100 Hz) or ripple (100-150 Hz) band based on its peak power frequency. In total, over 6000 electrodes recording field potential from cortical and subcortical structures in over 30 patients were used in this study. On average, 4.2% of electrodes in any one patient showed induced responses in low gamma band, 11.1% in the high gamma, and 13.6% in the ripple band during word presentation. Cluster analysis revealed two distinct populations detected on these active electrodes: majority of them (72.4 - 81.6 %) had discrete characteristic narrowband frequency with maximum bandwidth less than 40 Hz (14.0 - 26.6 Hz mean cluster centroid). In contrast, the remaining detections showed broad bandwidth above 40 Hz (52.8 - 78.5 Hz mean cluster centroid) characteristic of asynchronous power increases. The broadband power increases had significantly shorter duration and higher amplitude than the narrowband oscillations. Relative contribution of the two high frequency activities to subsequent

memory effect and to the physiological response to 50 Hz stimulation were also assessed. Our results show that the induced high frequency responses recorded during memory tasks are comprised of two distinct types of brain activity. These show qualitative and quantitative differences, suggesting specific physiological origins and contributions to cognitive processing. We propose that the broadband power increases reflect asynchronous neural activities, whereas the induced oscillations are generated by coordinated neuronal assemblies, which present viable targets for the development of novel neuromodulation technologies.

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Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 719.04/Y24

Topic: F.01. Human Cognition and Behavior

Support: DoD Contract: N66001-14-2-4032

Title: Biomarkers of human memory encoding during spatial navigation

Authors: ***T. J. COFFEY**[1], J. MILLER[2], S. LEE[3], M. SPERLING[4], A. SHARAN[5], G. WORRELL[6], B. BERRY[7], B. JOBST[8], K. DAVIS[9], T. LUCAS[9], R. GROSS[10], S. DAS[11], J. STEIN[9], B. LEGA[12], D. RIZZUTO[13], J. JACOBS[14];
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Abstract: The medial temporal lobe has been jointly implicated in both spatial and episodic memory function, largely on the basis of separate studies with different sets of participants. Here we analyse the brain signals that predict accurate encoding of spatial and episodic memories in a unified dataset, allowing us to directly compare the neural basis of these functions for the first time. Our studies examine the behavior and electrophysiology of neurosurgical patients with

intractable epilepsy using intracranial electrodes. In our spatial memory task, patients performed a virtual navigation task in which they had to remember the locations of hidden objects. We found statistically significant changes in the time-frequency spectra related to good memory in individual subjects across brain regions and frequency bands. In the hippocampus, successful memory encoding was marked by significant increases in power for the low-theta (1-3Hz) and high theta (3-9) Hz bands and reduction in power in the HFA (70-200Hz) band. Separately analyzing memory for easy and hard trials, we found distinct memory biomarkers for hard and easy memory trials in the low and high theta bands, as well as in the HFA range. We compared these findings with the memory signals identified in an episodic memory task, which revealed significant differences between the brain signals that underlie spatial and episodic memory. Overall, these results demonstrate that medial temporal structures support both spatial and non-spatial memory, but with different electrophysiological patterns underlying each of these behaviors.

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Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 719.05/Y25

Topic: F.01. Human Cognition and Behavior

Support: DoD Contract: N66001-14-2-4032

Title: Characterization of neural dynamics in response to electrical stimulation for designing a closed-loop control architecture for memory enhancement

Authors: *B. MAHMOUDI[1], R. GROSS[1], M. KAHANA[2];
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Abstract: The optimal control of neurostimulation is a joint optimization problem in the neural state- stimulation action space to maximize the probability of recall over a certain time horizon. The state vector is a representation of direct measurements of multi-channel Local Field Potential (LFP) time series. Our goal is to design an adaptive learning control system for automatic optimization of the spatio temporal distribution of the neurostimulation. One of the

key steps in designing the architecture of the control system is characterizing the effect of stimulation on the dynamics of LFP signals (controllability) during memory tasks. Towards analyzing the controllability of the state vector using electrical stimulation and eventually improving the objective function, we analyzed the transient effect of stimulation on the dynamics of the LFP signals. The LFP time series were partitioned into three different segments, with reference to the stimulation epochs, namely inter-stim, post-stim and stim segments. The inter-stim refers to a segment of the LFP signal that is temporally between two consecutive stimulation epochs. The post-stim refers to a segment of the signal that was recorded immediately after the stimulation. Finally, the stim segment refers to the recording during the stimulation. We analyzed the effect of stimulation on the duration of the post-stim segment. This duration corresponded to the settling time for the neural states to return back to base-line after stimulation. A moving window with variable length was used to detect the state transition to base-line after stimulation. The separability between the inter-stim and post-stim segments was measured probabilistically using a Support Vector Machine (SVM) classifier. By changing the length and the offset of the post-stim window, the SVM was trained to discriminate between the post-stim and the inter-stim states. The offset of the stimulation was detected automatically and was assigned as the reference point in time to define the starting point of the post-stim window. The results of classification between the post-stim and inter-stim groups suggested that electrical stimulation at 50 Hz induced a transient pattern in the LFP that distinguished the post-stim state from the baseline inter-stim state. The classification results using a window in time that progressively grew by 100 ms was relatively uniform across multiple window sizes, therefore, we selected the window size of 500 ms to study the effect of offset. A transition point around 1 sec was detected beyond which the statistics of the LFP signal returned back to the baseline, however, the decline point was not uniform across multiple recording sites.

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Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 719.06/Y26

Topic: F.01. Human Cognition and Behavior

Support: DoD Contract: N66001-14-2-4032

Title: Electrical stimulation in the medial temporal lobe alters memory encoding

Authors: *J. JACOBS[1], T. COFFEY[2], J. MILLER[2], S. LEE[3], M. SPERLING[4], A. SHARAN[4], A. ASADI-POOYA[4], G. WORRELL[5], B. BERRY[5], B. JOBST[6], K. DAVIS[7], T. LUCAS[7], R. GROSS[8], S. DAS[9], J. STEIN[7], D. RIZZUTO[10];
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Abstract: The hippocampus and entorhinal cortex have long been implicated in memory encoding processes from lesions and neuroimaging studies. Here we used electrical stimulation to directly test the role of these structures in memory encoding. Neurosurgical patients with implanted electrodes performed a version of Morris's water maze experiment, which we customized to include electrical stimulation at 50 Hz in deep brain structures during the period when they encoded each item's location. We assessed the effect of stimulation on memory encoding efficiency by comparing navigation accuracy between items where the patients received stimulation during encoding compared to non-stimulated items. Across- and within-subject analyses revealed that stimulation significantly impaired the accuracy of memory encoding. These findings establish the hippocampus and entorhinal cortex as playing a critical causal role in memory encoding by showing that stimulation in these regions affects memory performance. Going forward, they suggest that the goal of using electrical stimulation to improve memory will require more advanced stimulation protocols.

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Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

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Program#/Poster#: 719.07/Y27

Topic: F.01. Human Cognition and Behavior

Support: DoD contract: N66001-14-2-4032

Title: Frequency-specific network connectivity during encoding predicts subsequent free recall

Authors: *C. S. INMAN[1], M. J. JUTRAS[2], J. T. WILLIE[1], B. C. JOBST[3], M. R. SPERLING[4], A. D. SHARAN[4], T. H. LUCAS[5], K. A. DAVIS[5], D. S. RIZZUTO[5], R. E. GROSS[1]; [1]Neurosurg., Emory Univ., Atlanta, GA; [2]Univ. of Washington, Seattle, WA; [3]Dartmouth-Hitchcock Med. Ctr., Lebanon, NH; [4]Thomas Jefferson Univ., Philadelphia, PA; [5]Univ. of Pennsylvania, Philadelphia, PA

Abstract: The medial temporal lobe (MTL) structures, including the hippocampus and surrounding cortical regions, play an essential functional role during the encoding of episodic memories. Evidence from electrophysiological studies of rodents, non-human primates, and human patients suggest that memory formation is accomplished through coordinated activity between MTL structures, like the hippocampus, and neocortical regions. However, the physiological, network-level mechanisms underlying these MTL-cortical interactions during episodic memory encoding are not yet clear. Large-scale functional network analysis using graph theory in human intracranial EEG (iEEG) data can help reveal the central network-level role the hippocampus and other MTL regions play during episodic memory encoding. We measured local field potentials in 9 patients undergoing iEEG monitoring for treatment-resistant epilepsy while patients encoded a series of word lists. We constructed field-field coherence matrixes between all pairs of electrodes in the recorded network for each patient across multiple frequency bands between 1 and 100 Hz for words that were later recalled or not recalled. We then analyzed all electrodes (nodes) in each patient's coherence matrixes with two network measures of node centrality. Specifically, we estimated the global node centrality for each electrode with the measure "node betweenness" and the local centrality with "node degree." Higher centrality for a specific region is indicative of the region's role in facilitating functional integration in the network. Both centrality metrics were normalized for different electrode counts per participant. Finally, we tested whether the node centrality for hippocampal electrodes within each patient's coherence network predicted subsequent free recall. We found that hippocampal electrodes showed both higher node betweenness and node degree centrality in each patient's recorded network when encoding a word that would be later recalled than not recalled ($p<0.05$) for coherence networks specific to gamma activity (50-100 Hz), but not other oscillatory frequencies. This finding provides further evidence that the hippocampus is a hub of the episodic memory encoding network and may support binding of encoded features into an enduring long-term memory. This large-scale network characterization of the role MTL structures play in encoding may be useful in guiding the selection of sites for stimulation to modulate network activity and episodic encoding. Future analyses will explore these network-level subsequent memory effects in sub-regions of the hippocampus and other hypothetical encoding network nodes.

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Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Topic: F.01. Human Cognition and Behavior

Support: DoD Contract N66001-14-2-4032

Title: Modulation of macaque hippocampal activity with entorhinal and septal stimulation

Authors: *A. G. RICHARDSON[1], P. K. WEIGAND[1], D. S. RIZZUTO[2], M. J. KAHANA[2], T. H. LUCAS[1];
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Abstract: Deep-brain stimulation (DBS) has been proposed as a treatment for impaired hippocampal-dependent memory formation. However, little is known about how DBS affects hippocampal activity in primates, including humans. Here, we sought to modulate oscillations in the macaque hippocampus with stimulation of two major upstream areas: the entorhinal cortex (EC) and the medial septum (MS). Two rhesus macaques were chronically implanted with three clinical DBS arrays (Ad-Tech Medical) targeting the hippocampus, EC, and MS. Experiments were conducted with the animals lightly sedated with ketamine. A subset of the experiments was subsequently repeated with the second animal awake and freely behaving. The main finding of EC stimulation experiments was that evoked hippocampal responses were strongly dependent on ongoing hippocampal gamma activity. Single EC stimulus pulses evoked a short-latency potential in the hippocampus only 15-35% of the time. The 30-90 Hz power in the 50 ms prior to the stimulus was significantly higher in trials with evoked potentials versus without (t test, $p < 1e-6$). Closed-loop, gamma-triggered EC stimulation increased the evoked potential rate to 60-95%. The state-dependence of hippocampal activation was a prominent feature only of EC stimulation, not MS stimulation. The main finding of MS stimulation experiments was that gamma-frequency stimulus trains initiated gamma-frequency oscillations in the hippocampus. Entrainment occurred only over the low-gamma frequency range, 30-60 Hz. Entrained hippocampal oscillations typically started on the 3-4 pulse of the train and continued for 2-3 cycles after the end of the stimulus train. The post-stimulus amplitude of the evoked hippocampal oscillations was 4-6 times bigger than the size of the single-pulse evoked potential. Thus the MS-driven hippocampal response exhibited both entrainment and resonance. In contrast, entrainment and resonance were not features of the hippocampal response to EC stimulus trains. The hippocampal response frequency was independent of EC train frequency. And the post-stimulus response amplitude was approximately the same for trains and single

pulses. Gamma oscillations are thought to be critical to information flow in the hippocampus and to memory formation and retrieval. The present work demonstrates in primates with clinical DBS electrodes how to initiate hippocampal gamma rhythms with MS stimulation and how to time EC stimulation to those rhythms to maximize the hippocampal response.

Disclosures: A.G. Richardson: None. **P.K. Weigand:** None. **D.S. Rizzuto:** None. **M.J. Kahana:** None. **T.H. Lucas:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 719.09/Y29

Topic: F.01. Human Cognition and Behavior

Support: Department of Defense Contract: N66001-14-2-4032

Title: Multivariate pattern analysis of the neural correlates of memory and lexical semantics measured using invasive electrocorticography during a free recall task

Authors: *A. C. CONNOLLY[1], J. DAMIANOS[2], P. HORAK[1], M. SPERLING[3], A. ASADI-POOYA[3], G. WORRELL[4], B. BERRY[4], K. DAVIS[5], R. GROSS[6], B. LEGA[7], D. RIZZUTO[8], M. KAHANA[8], B. JOBST[1];
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Abstract: There are several known predictors of recall success in freerecall tasks, i.e., serial position, context retrieval, semantic clustering,and lexical frequency. Less is known, however, about the inherent memorablenessthat can be predicted based on a word's meaning. Given a list of mundane itemslike “table”, “rock”, and “dog” with more attention grabbing words like“knife”, “bullet”, or “shark”, we predict that the latter sort will be morereadily remembered. This semantic saliency hypothesis proposes that recallprobability for words can be predicted based on a semantic model. We analyzeddata from a large free-recall database (pyFR). Using Google' s semantic model(word2vec), we fit a regression model to predict recall probability for wordsin pyFR based on model vectors. A regression model was fit based on vectorsfrom all words but one, then recall probability of the left-out word waspredicted based on its vector. Correlation between the actual and predictedrecall probabilities was strong (r = .53), controlling

for other factorsincluding word frequency. Cluster analysis based on model vectors suggests asemantic dichotomy segregating animate from inanimate nouns; furthermore,recall probability of animate nouns was significantly greater than forinanimate nouns, suggesting factors related to animacy may predict recall. Weinvestigated neural correlates of animacy using invasive electrocorticography(ECoG) during a free recall task in a new group of patients (RAM_FR1: record only, andRAM_FR2: during which medial temporal lobe structures were electricallystimulated, N = 19). We coded nouns as animate or inanimate. Using patterns ofECoG magnitude across electrodes, we trained pattern classifiers for animateversus inanimate. We were able to decode animacy above chance in thenon-stimulated group, but not for the stimulated group, but the differencebetween groups was not significant. Accuracy across all subjects wassignificantly better than chance. Our findings suggest (1) semantics of wordsin a free recall task can contribute to the likelihood of recall, (2) theanimacy of nouns may be related to recall, and (3) the biomarkers of semanticrepresentations for animacy can be reliably measured using ECoG.

Disclosures: **A.C. Connolly:** None. **J. Damianos:** None. **P. Horak:** None. **M. Sperling:** None. **A. Asadi-Pooya:** None. **G. Worrell:** None. **B. Berry:** None. **K. Davis:** None. **R. Gross:** None. **B. Lega:** None. **D. Rizzuto:** None. **M. Kahana:** None. **B. Jobst:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: DoD Contract: N66001-14-2-4032

NINDS Intramural Research Program

Title: Examining biomarkers of memory encoding and effects of electrical stimulation in human subjects performing paired associates learning

Authors: *J. H. WITTIG, JR[1], R. YAFFE[1,2], S. INATI[1], G. WORRELL[3], T. LUCAS[4], A. SHARAN[5], D. RIZZUTO[4], K. ZAGHLOUL[1,4];
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Abstract: We have examined the electrophysiological biomarkers of recall performance during paired associates learning in a cohort of patients that have subdural electrodes placed for seizure monitoring. Participants were asked to memorize (encode) a list of 6 pairs of words, and approximately 45 seconds later they were prompted to recall each pair when one of the words from the pair was displayed on a computer monitor. Each testing session consisted of 25 such lists, and participants often completed 2 or more sessions while intracranial EEG signals were recorded from each subdural electrode. For each electrode, we compared the spectral power of correctly remembered versus forgotten word-pairs by evaluating (1) mean power differences during encoding, (2) mean power differences during recall, and (3) trial-by-trial similarity, or reinstatement, between encoding and recall. A subset of the cohort of patients (3 participants) performed additional paired associates learning sessions while they received subdural electrical stimulation time locked to the encoding period (10 of 25 lists) or the recall period (10 of 25lists). Electrical stimulation during encoding and/or recall caused no change in the percentage of correctly recalled word-pairs for any of the 3 participants; the only behavioral changes we observed were an increase in response time in 1 participant during recall stimulation, and an increase in the percentage of intrusion errors in another participant during recall stimulation. In this preliminary report, we assess the predictive power of biomarkers identified during non-stimulation sessions on the existence or absence of any behavioral effect of electrical stimulation.

Disclosures: J.H. Wittig: None. **R. Yaffe:** None. **S. Inati:** None. **G. Worrell:** None. **T. Lucas:** None. **A. Sharan:** None. **D. Rizzuto:** None. **K. Zaghloul:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

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Program#/Poster#: 719.11/Y31

Topic: F.01. Human Cognition and Behavior

Support: DoD Contract N66001-14-2-4032

Title: Interictal epileptiform discharges impair performance in a word recall task

Authors: *P. HORAK[1], A. ROBBINS[1], A. CONNOLLY[1], S. MEISENHELTER[1], M. TESTORF[2], M. SPERLING[3], A. ASADI-POOYA[3], G. WORRELL[4], B. BERRY[4], K. DAVIS[5], R. GROSS[6], B. LEGA[7], D. RIZZUTO[8], B. JOBST[1,9];
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Abstract: Cognitive deficits, particularly related to memory loss, are one of the most crippling side-effects of epilepsy, sometimes causing patients as much hardship as the seizures themselves. There is a pressing need to understand memory loss in epilepsy in order to identify better interventions. Characterizing the factors behind memory loss, such as seizures or interictal epileptiform discharges (IEDs), is a crucial step in this direction. Past studies have demonstrated that hippocampal IEDs may interfere with memory maintenance and retrieval in humans. In the present study, we use an automated detector to facilitate marking and analyzing IEDs in a larger set of brain regions and memory tasks. We analyzed electrocorticography data from 21 subjects in two tasks that were administered as part of the Restoring Active Memory project (RAM). In the first experiment, subjects studied a list of words and later recalled them. In the second experiment, subjects tried to remember the spatial location of objects in a virtual environment. For each paradigm, we analyzed the relationship between memory performance and IEDs in the hippocampus, parahippocampal gyrus, entorhinal cortex, and middle temporal gyrus. We found no significant effect of IEDs on performance in the spatial task. Meanwhile, IEDs that occurred in the middle temporal or parahippocampal gyrus during the study portion of the free recall task showed a significant association with words that were later forgotten ($p<0.0001$ and $p<0.05$ respectively). These results suggest that middle temporal IEDs may hinder mental processes specific to word encoding in the free recall task. This would be consistent with existing research that implicates the region in semantic processing and perception.

Disclosures: **P. Horak:** None. **A. Robbins:** None. **A. Connolly:** None. **S. Meisenhelter:** None. **M. Testorf:** None. **M. Sperling:** None. **A. Asadi-Pooya:** None. **G. Worrell:** None. **B. Berry:** None. **K. Davis:** None. **R. Gross:** None. **B. Lega:** None. **D. Rizzuto:** None. **B. Jobst:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Topic: F.01. Human Cognition and Behavior

Support: DoD Contract: N66001-14-2-4032

Title: Using a detailed computational model of behavior to decompose the subsequent memory effect in free recall

Authors: *Z. TIGANJ[1], J. M. DI LASCIO[1], J. F. BURKE[2], Y. EZZYAT[2], P. B. SEDERBERG[3], M. J. KAHANA[2], D. RIZZUTO[2], M. W. HOWARD[1];
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Abstract: In the free recall task, participants recall as many words as they can from a list in the order the words come to their mind. Subsequent memory effect (SME) analyses contrast the neural biomarkers during encoding of words that are later recalled to the biomarkers during encoding of words that are not recalled. However, in free recall there are many reasons why a particular word might be successfully recalled. For instance, the word might be particularly easy to recall due to its position in the list, or because it is semantically related to other words in the list. In order to separate the effect of these exogenous variables on the SME, we used a detailed computational model to identify the likelihood that each study word would be free recalled. Power at several frequency bands at three time points was measured from ECoG electrodes at a variety of locations in patients. We then regressed power onto the likelihoods provided by the behavioral model and compared the results to a multivariate SME. This enabled us to subdivide the SME into a part that aligns with the likelihood of a word being recalled, and a residual that is orthogonal to the likelihoods. The former component is the part of the SME attributable to all the exogenous variables incorporated into the behavioral model (serial position, semantics, etc.); the latter presumably includes biomarkers of endogenous variables that predict good memory encoding. The general strategy of using detailed computational models of memory to inform neurophysiological data analyses could yield dramatic insights into the processes of memory retrieval.

Disclosures: Z. Tiganj: None. **J.M. Di Lascio:** None. **J.F. Burke:** None. **Y. Ezzyat:** None. **P.B. Sederberg:** None. **M.J. Kahana:** None. **D. Rizzuto:** None. **M.W. Howard:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1157432

NIH Grant UL1 TR000445

Vanderbilt University Discovery Grant

Title: Decoding episodic retrieval processes: frontoparietal and medial temporal lobe contributions to free recall

Authors: *J. E. KRAGEL, S. M. POLYN;
Vanderbilt Univ., Nashville, TN

Abstract: Neuroimaging studies of recognition memory have identified distinct patterns of cortical activity associated with two sets of cognitive processes: Recollective processes supporting retrieval of information specifying a probe item's original source are associated with activation of the hippocampus, ventral posterior parietal cortex, and medial prefrontal cortex. Familiarity processes supporting the correct identification of previously studied probes (in the absence of a recollective response) are associated with activity in anterior medial temporal lobe (MTL), and lateral prefrontal and dorsal posterior parietal regions. Here, we address an open question in the cognitive neuroscientific literature: To what extent are these same neurocognitive processes engaged during an internally directed memory search task like free recall? We recorded functional magnetic resonance imaging (fMRI) activity while participants performed a series of free recall and source recognition trials, and used a combination of univariate and multivariate analysis techniques to compare neural activation profiles across the two tasks. Univariate analyses showed that posterior MTL regions were commonly associated with recollective processes during source recognition, and with free-recall responses. Prefrontal and posterior parietal regions were commonly associated with familiarity processes and free-recall responses, whereas anterior MTL regions were only associated with familiarity processes during recognition. In contrast with the univariate results, free-recall activity patterns characterized using multivariate pattern analysis did not reliably match the neural patterns associated with recollective processes. However, these free-recall patterns did reliably match patterns associated with familiarity processes, supporting theories of memory in which common cognitive mechanisms support both item recognition and free recall.

Disclosures: **J.E. Kragel:** None. **S.M. Polyn:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Support: NSF Grant 1157432

Vanderbilt Discovery Grant

Title: Oscillatory correlates of enhanced memorability following a shift in the perceptual modality of studied material

Authors: *J. D. MCCLUEY, S. M. POLYN;
Psychology, Vanderbilt Univ., Nashville, TN

Abstract: The human memory system interacts with an ever-changing perceptual environment, and the perceptual properties of new information can shape that information's mnemonic fate. Experimentally, shifts in perception to a salient stimulus have been shown to enhance memorability for post-shift items (Murdock & Walker, 1969). Despite this work, the neural signals that reflect this enhanced memorability at perceptual boundaries remain to be characterized. Sederberg et al. (2006) described global shifts in the oscillatory topography elicited by studied items at early vs. late serial positions, and showed distinct subsequent memory responses for items of each type. One interpretation of this oscillatory shift is that the presentation of the first few list items reflects a perceptual shift relative to experiences prior to the start of the current list, which enhances the memorability of the first few items. In a scalp EEG experiment, we manipulated the presentation modality (auditory vs. visual) of studied material to elicit a mid-list perceptual shift that enhances the memorability of the post-shift item. These post-shift items elicited an oscillatory response which showed enhanced delta activity (2-4 Hz) and diminished alpha and beta activity (10-26 Hz) relative to non-shift items. We examined how these signals changed with the subsequent memory status of the post-shift item, and contrast these effects with list position effects described by Sederberg et al. (2006). In addition, we used a retrieved-context model of memory search to investigate the potential cognitive mechanisms underlying the oscillatory signatures characterized by this study.

Disclosures: J.D. McCluey: None. **S.M. Polyn:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: BYU Graduate Studies Fellowship

Title: The effects of exercise on the neural correlates of pattern separation

Authors: *B. KIRWAN[1], S. SPENCER[2], M. I. NASH[3];
[1]Psychology, [2]Neurosci., [3]Brigham Young Univ., Provo, UT

Abstract: While there are many known benefits of regular physical activity (PA), including lower rates of mortality and morbidity, the majority of individuals in the United States do not engage in the recommended levels of fitness. Furthermore, there have been a limited number of studies evaluating the effect PA may have on cognitive abilities and neurological components and none have evaluated what effect the recommended levels of fitness may have on these areas. The current study evaluated differences between individuals with varying levels of PA using functional magnetic resonance imaging (fMRI) during the performance of a mnemonic discrimination task meant to tax pattern separation processes. Differences in diffusion tensor imaging (DTI) were also examined. Individuals were separated into three groups based on established PA patterns. These groups consisted of sedentary individuals, individuals who did not meet American Heart Association (AHA) recommendations of PA, and individuals who met or exceeded AHA recommendations. Twenty individuals (10 men and 10 women) were recruited for each group. The mnemonic discrimination task consisted of a series of color photographs of everyday objects shown one at a time. For each image, participants indicated whether the object was “new” (objects shown once_foils), “old” (objects shown twice_repeats), or “similar” (objects consisting of paired images that were visually and conceptually similar_lures). It was hypothesized that participants with self-reported higher levels of PA would have a greater bias toward pattern separation both behaviorally and in the fMRI activation of the CA3/dentate gyrus subregions of the hippocampus than those with lower fitness levels and sedentary individuals. Furthermore, it was predicted that those with higher levels of PA would have increased white matter integrity in the cingulum, uncinate fasciculus, and temporal lobe pathways. Significant differences were found in white matter integrity (axial diffusivity or AD) in bilateral cingulum, the left temporal middle gyrus, and the right uncinate fasciculus. Surprisingly, no behavioral or functional differences were found across groups, which may have been influenced by the task demands and small group differences in recorded activity level.

Disclosures: **B. Kirwan:** None. **S. Spencer:** None. **M.I. Nash:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Topic: F.01. Human Cognition and Behavior

Support: BYU Graduate Studies Fellowship

BYU MRI RF Seed Grant

Title: An fMRI investigation on how testing format affects performance on a pattern separation task

Authors: *M. ANDERSON, C. DOXEY, B. KIRWAN, M. NASH;
Brigham Young Univ., Provo, UT

Abstract: Many published studies aimed at investigating memory specificity or mnemonic discrimination use a recognition memory paradigm meant to tax pattern separation processes. These tasks require participants to view images of everyday objects, one at a time, and classify them as "new" if it is the first time the participant is viewing the image in the study (New), "old" if the image is an exact duplicate of an image already seen (Repeat), or "similar" if the image is similar to a previously viewed image but not exactly the same (Lure). Participant responses to Lure stimuli are used to evaluate memory specificity. Lures classified as "similar" (Lure Correct Rejection) indicate a higher degree of memory specificity compared to Lures classified as "old" (Lure False Alarm). This memory specificity paradigm has been used by researchers in mainly two different formats: a study-test design and a continuous recognition design. In the study-test design, participants complete a study phase, in which they view the stimuli and classify them as belonging indoors or outdoors, followed by a test phase. Only during the test phase are the participants asked to classify the images as "new", "similar", or "old". In the continuous recognition design participants view the stimuli and classify them as "new", "old", or "similar" without any previous exposure (i.e. there is no study phase, only a testing phase). In this study, we evaluated the behavioral differences of these two paradigms and used functional magnetic resonance imaging (fMRI) to assess the neurological correlates. The study-test condition consisted of 49 participants and the continuous condition consists of 52 participants. Using a pattern separation score (calculated by subtracting the proportion of "similar" responses to New stimuli from the proportion of "similar" responses to Lures to correct for possible response bias), the continuous recognition group preformed significantly better ($p < .001$) compared to the study-test group in correctly identifying Lures (similar images), indicating a higher degree of memory specificity. However, the overall pattern of fMRI activation in the hippocampus and in the cortex was similar between the two groups.

Disclosures: **M. Anderson:** None. **C. Doxey:** None. **B. Kirwan:** None. **M. Nash:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: BYU MRI RF Seed Grant

Title: An fmri investigation of the impact of sleep on pattern separation

Authors: *C. DOXEY[1], B. KIRWAN[2];
[2]Neuroscience/Psychology, [1]Brigham Young Univ., Provo, UT

Abstract: The unique anatomy of the hippocampus, and particularly the hippocampal subfields, makes it especially suited for performing complex computations while forming and retrieving long-term declarative memory representations. One such computational process, known as pattern separation, establishes orthogonal or non-overlapping memory representations in order to discriminate between similar memories. In pattern separation, memory representations are established to allow individuals to successfully recall specific details of previously viewed stimuli and discriminate between similar memory representations. Pattern completion is a complementary process that allows for previously stored memory representations to be reactivated from degraded or noisy cues, resulting in generalization from similar memory representations. The processes of pattern separation and pattern completion have been widely studied in a variety of contexts and models. Human studies have relied on memory specificity paradigms that tax pattern separation, but the effects of sleep on memory specificity have yet to be elucidated. Studies have implicated that consolidation that occurs during sleep improves declarative recognition memory, so we hypothesized that sleep would have a similar effect on memory specificity. We scanned 50 participants using fMRI who were assigned to either a sleep or a wake condition. Participants in both groups studied pictures of random, everyday objects and were then tested immediately on their memory for half of the stimuli while undergoing fMRI scanning. During the testing portion, participants were shown exact repeats, similar lures, and completely new stimuli, and were asked whether each object seen was “old”, “similar”, or “new”. They were brought back to the scanner 12 hours later where they were tested on the remaining objects. Participants in the wake group performed the study and immediate test in the morning and the delayed test in the evening with no sleep between. Participants in the sleep group performed the study and immediate test in the evening and the delayed test in the morning following a night’s sleep. Participants in the sleep group were better at labeling lure stimuli as “similar” compared to participants in the wake group. Interestingly, this sleep effect was not found when analyzing recognition memory in broad terms. Behavioral results were correlated with the simultaneous fMRI data in which differences were found between groups. The implications of hippocampal subfield activity on memory specificity processing will be discussed.

Disclosures: **C. Doxey:** None. **B. Kirwan:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Topic: F.01. Human Cognition and Behavior

Support: T32-MH067564

P50-MH094263

Title: Neural correlates of repetitive versus randomly sequenced learning of context-dependent associations

Authors: ***D. R. O'YOUNG**, J. L. VOSS;
Northwestern Univ., Chicago, IL

Abstract: Associative learning occurs within specific contexts that govern the appropriate behavioral expression of memory (e.g. mixing sucrose into buffer solution in lab, but into coffee at home). Human and animal research suggests that hippocampal and prefrontal regions are especially involved in flexible, rule-based learning rather than inflexible, stimulus-response learning. Here, we investigated neural correlates of context-dependent association learning under conditions designed modulate the involvement of flexible, rule-based learning. Trials were presented in one of four quadrants (e.g. contexts). Given a Cue object, subjects indicated if a subsequent Target object was associated or not. Cue-Target associations were varied by context such that Targets could be classified as Associated-Match (AM, associated with cue and context), Associated-Nonmatch (AN, associated with cue in other contexts), or Unassociated (UA, never associated with cue). Each subject (N=20) performed the task during two sessions, including either (i) randomized trial sequences to promote learning all associations concurrently and the use of rule-based retrieval of associations or (ii) repetitive trial sequences to promote learning each association individually and the use of stimulus-response associations. During memory testing, accuracy was high and did not differ for randomized versus repetitive learning sequences, indicating successful learning and expression of memory for both conditions. ERPs in response to cues included positive potentials from 250-600 ms at frontal sites with greater amplitudes for repetitive learning, suggesting greater preparatory recall in response to cues (rather than to targets) in this condition. In contrast, relative to repetitive learning, randomized learning led to enhanced positive potentials (250-500 ms) for AN targets versus UA targets,

suggesting utilization of contextual information to resolve ambiguities in associative recall in response to targets rather than cues. For both repetitive and randomized conditions, AM targets resulted in enhanced positive potentials (250-600 ms) relative to AN and UA targets, suggesting no nonspecific differences in neural correlates of successful memory retrieval for these conditions. These results suggest that different modes of learning, distinguished only by trial order, engage different brain systems during subsequent expression of memory. Results are interpreted regarding distinctions between human and animal memory research, which often differ in consistency and predictability of trial order during training, potentially promoting involvement of different brain systems.

Disclosures: D.R. O'Young: None. **J.L. Voss:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Topic: F.01. Human Cognition and Behavior

Support: NIMH P50-MH094263

NINDS NS083340

NRSA F32NS087885

Title: Targeted enhancement of hippocampal-cortical networks alters neural correlates of object-location association memory

Authors: *A. NILAKANTAN, D. BRIDGE, E. GAGNON, J. WANG, J. VOSS;
Med. Social Sci., Northwestern Univ., Chicago, IL

Abstract: We have previously shown that multi-day repetitive transcranial magnetic stimulation applied to fMRI-defined sites of hippocampal-cortical resting-state networks enhances network connectivity and associative memory. Here, we aimed to extend these findings by examining neural correlates of changes in associative memory retrieval following hippocampal-cortical network-targeted stimulation. In a counter-balanced design over two separate weeks, participants (n=8) completed five consecutive days of network-targeted stimulation (using individualized parietal locations within resting-state fMRI connectivity networks of the hippocampus) versus five consecutive days of sham stimulation (applied to vertex). EEG was recorded during an object-location memory task 24 hours prior to the first stimulation session and 24 hours

following the final stimulation session. Participants studied objects in distinct locations and later recalled object locations during the memory test. Effects of stimulation were assessed for a continuous measure of spatial recall (low precision to high precision placement). High-precision memories selectively improved following network-targeted stimulation relative to sham, suggesting that memory improvements were specific to hippocampal-mediated recollective processing. Furthermore, event-related neural correlates of high-precision recall changed due to stimulation, with greater amplitudes of late-positive activity for parietal electrodes that are normally observed for recollection (associated with oscillatory power increases in the delta and theta bands). These findings demonstrate that the enhancement of hippocampal resting-state fMRI networks caused by targeted noninvasive stimulation translates into enhanced neural correlates of successful memory retrieval.

Disclosures: **A. Nilakantan:** None. **D. Bridge:** None. **E. Gagnon:** None. **J. Wang:** None. **J. Voss:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Topic: F.01. Human Cognition and Behavior

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NIH Grant R01MH062500

Title: Dominance provides structure to episodic memories

Authors: ***D. J. BRIDGE**, J. L. VOSS;
Med. Social Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Of the many elements that comprise an episode, are any particularly important for coherent memory binding? It is possible that all episode elements are bound equally and recriprocally, and therefore equally support memory retrieval. However, we reasoned that there is structure to memory representations, such that binding is unequally distributed among event elements based on dominance. We hypothesized that active retrieval causes select elements to attain dominance, which enables these elements to be disproportionately bound with other less-dominant elements. We tested this hypothesis using a multi-element episodic memory task. Subjects (N=16) studied three objects at specific locations. After a brief delay, subjects selected

one object and recalled its associated location. Memory was later tested for the other, less-dominant objects. When high-dominance objects served as reminder cues, retrieval of less-dominant objects was significantly more accurate than when less-dominant objects were reminder cues. Specifically, memory of the episode configuration was enhanced when a dominant cue was used as a reminder cue relative to a less-dominant cue. Binding of all objects was therefore strongest to the high-dominance object. Further, dominance binding was associated with enhanced fMRI activity in the hippocampus, suggesting that the hippocampus is involved in disproportionately binding dominant elements to less-dominant elements. Eye movements recorded during the study phase provided mechanistic evidence for disproportionate binding with the dominant objects. A control condition involving passive manipulation of objects rather than active retrieval established the specificity of these dominance effects on behavior and fMRI correlates. Active retrieval therefore selectively increases dominance of specific episode elements and thereby organizes episodic memory. Binding is not equipotent, but rather dominant elements have disproportionately strong binding and therefore serve as effective retrieval cues for coherent episodes.

Disclosures: D.J. Bridge: None. **J.L. Voss:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 719.21/Y41

Topic: F.01. Human Cognition and Behavior

Support: NINDS T32NS047987

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NIMH P50-MH094263

Title: Memory awareness modulation due to theta-burst stimulation of distinct prefrontal cortical networks

Authors: *A. J. RYALS, J. L. VOSS;
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Abstract: Evidence from neuroimaging and lesion studies suggests that subjective memory awareness may involve anterior prefrontal cortical regions acting in concert with the hippocampus. We used theta-burst transcranial magnetic stimulation (TBS) to temporarily

modulate dorsolateral versus frontopolar prefrontal cortex to test for distinct causal roles in memory awareness in healthy individuals. TBS targets were identified in frontopolar cortex (FPC), dorsolateral prefrontal cortex (DLPFC), and sham (vertex) locations, and were stimulated bilaterally in each of three distinct experimental sessions over the course of one week using a within-subjects design (N=18). FPC locations were found to have greater resting-state fMRI connectivity with hippocampus relative to DLPFC locations, further suggesting that these locations might have critical importance for memory awareness. Associative recognition accuracy did not differ based on stimulation location. In contrast, frontopolar stimulation significantly influenced several measures of memory awareness. During study, judgment of learning accuracy increased, such that lower ratings were given to items that were subsequently forgotten selectively following frontopolar TBS. Confidence ratings during test were also higher for correct trials following frontopolar TBS. Finally, the trial-by-trial correspondence (i.e., gamma correlation) between overt performance and subjective awareness during study demonstrated a linear increase across control, dorsolateral, and frontopolar TBS locations, supporting a proposed rostrocaudal hierarchy of prefrontal contributions to memory awareness. These findings indicate that frontopolar cortex contributes causally to memory awareness, which was selectively enhanced by anatomically targeted TBS. Effects of TBS on FPC versus DLPFC functional connectivity networks will be discussed in relation to the effects of TBS on memory awareness.

Disclosures: **A.J. Ryals:** None. **J.L. Voss:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 719.22/Y42

Topic: F.01. Human Cognition and Behavior

Support: The John Templeton Foundation

Title: Refresh my memory: Context information from episodic memory affects working memory maintenance

Authors: ***A. NOVICK**, A. M. BORNSTEIN, J. D. COHEN;
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Abstract: Humans rely on many kinds of memory to make decisions. Some memory processes, such as working memory (WM) and episodic memory (EM), have distinct neural substrates and

can be subject to distinct disruptions. For instance, when WM maintenance is hindered in simple short-term memory tasks- e.g., when one's ability to rely on a "phonological loop" is disrupted - EM is used to guide behavior. Here, we investigated whether we might see evidence of EM's effect on behavior even when WM is not disrupted. Episodic memory is known to carry information about the associative and temporal context at the time of encoding that is not present in working memory (Howard & Kahana 2002). Therefore, we sought signatures of the influence of encoding context on behavior. 55 subjects studied four word lists, each in a different "context" - a picture of a face or a natural scene. In addition to studying the words, subjects were asked to provide verbal descriptions of the pictures accompanying each list. After training, subjects completed a delayed match (or nonmatch) to sample task. On each trial, they viewed a target set, four words drawn from one of the previously learned lists. After an 18 second delay, subjects were shown a probe word, and asked whether that word was among the target set. Three kinds of probes were used: a word in the target set itself, a word from the same list as the target set, or a word from a different list. These tasks have previously been shown to rely on sustained WM representations. We aimed to test whether WM representations are supported by occasional refreshes from EM. We reasoned that, if EM were used to refresh those representations, we would see effects of the reinstated context from the time of learning. Specifically, we hypothesized that if context information were recalled from EM and present in WM, then the process of comparing the item on screen to those in working memory would be slowed if the probe was from the same list (context) as the targets. Alternatively, if WM representations were entirely self-sustaining, then no context information would be present, and same-list probes would be responded to equally as quickly as different-list probes. Our results matched the former pattern, with same-list probes significantly slowing responses relative to different-list or targets. Critically, the effect depended on how strongly a subject encoded the context: A more vivid verbal description of the context at encoding lead to a slower RT for same-list probes. This result is strongly supportive of the presence of episodic context content in working memory, and thus for a role for episodic memory in sustaining working memory representations.

Disclosures: **A. Novick:** None. **A.M. Bornstein:** None. **J.D. Cohen:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH100121

Title: Developing a neurocognitive model of memory integration

Authors: *N. W. MORTON, A. R. PRESTON;
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Abstract: Memories are not formed in isolation, but rather are learned in the context of a vast store of existing knowledge. As a result, one of the central problems faced by the memory system is resolving interference between similar memories. Recent research demonstrates that the brain can integrate new information with existing memories in a way that not only reduces interference, but also facilitates new learning. The hippocampus and areas of the prefrontal cortex (PFC) are critically involved in this process of memory integration; however, many questions remain about how these regions work together to combine information across related memories. We developed a modeling framework based on the temporal context model (TCM), describing how the processes of associative binding, memory reactivation, and integration with reactivated memories determine how new events are encoded. Within this framework, we tested a set of competing neurocognitive models that linked brain regions implicated in memory integration (hippocampus, medial PFC, and ventrolateral PFC) to cognitive processes specified by the model. We used a recently developed model-based fMRI technique that we term predictive model analysis (PMA) that uses trial-level measurements of brain activation to estimate the degree of engagement of each process involved in memory integration. The neurally informed models were then used to predict subsequent memory for overlapping experiences. Specifically, we used PMA to examine hippocampal and PFC contributions to a task in which participants learned pairs of images that overlapped with pairs learned previously. We found that ventrolateral PFC engagement could be used to estimate trial-level fluctuations in a model parameter reflecting encoding of individual associations, allowing better prediction of behavior compared to a model not informed by neural data. In contrast, activity in ventral temporal cortex allowed us to estimate the strength of reactivation of overlapping memories. Hippocampus and medial PFC activation was linked to a model parameter formalizing integration of temporal context across overlapping events, consistent with the idea that these regions play a specialized role in memory integration. Our results demonstrate the potential of using PMA to test hypotheses about links between cognitive processes and their neural substrates. Here, this novel method allowed us to disentangle the interrelated mechanisms through which hippocampus and PFC contribute to memory integration during new learning.

Disclosures: N.W. Morton: None. **A.R. Preston:** None.

Poster

719. Human Memory Processes: Encoding, Retrieval, and Pattern Separation

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Topic: F.01. Human Cognition and Behavior

Support: NIH AG034613

Title: The effect of task demands on activity in hippocampal subfields and MTL cortices in a pattern separation task

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Abstract: Several studies have reported activity in the combined dentate gyrus and CA3 (DG/CA3) subfields of the hippocampus that is consistent with pattern separation, or the orthogonalization of highly similar patterns of input. In CA1, a contrasting pattern of activity has been observed that is less sensitive to small changes and may track the degree of change. Here, we extend these findings by directly comparing the use of an incidental task with an intentional recognition memory task to evaluate the potential impact of the over memory task demands on activity. In a between-subjects design, participants either incidentally encoded everyday objects with an indoor/outdoor judgment or performed a continuous recognition task with old, similar, or new responses (the Mnemonic Similarity Task). Trials of interest included first presentations, exact repetitions, and similar lure items that were visually similar to a previously viewed item, but not exactly the same. We also included a perceptual discrimination baseline task. Throughout, signals consistent with pattern separation were observed in the DG/CA3, replicating prior results. In addition, both in the hippocampal subfields and in the MTL cortical regions, results were similar across intentional and incidental tasks. However, two caveats should be noted. First, the intentional task typically yielded more robust activity suggesting that task demands can be relevant. Second, pattern-separation related activity in the subfields and the MTL cortices differed as a function of how pattern separation was operationalized (using the recognition memory responses vs. using repetition suppression). These data highlight the importance of how one operationalizes computational concepts like pattern separation when it cannot be directly observed (e.g., using imaging techniques) and stresses the need for relating findings such as these that can be observed in humans with more direct evidence from animal models.

Disclosures: S.M. Stark: None. **C.E. Stark:** None.

Poster

720. Human Decision Making: Risk and Impulsivity

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NSF GRFP

Title: Propranolol influences reference-dependence in intertemporal choice

Authors: *K. M. LEMPERT[1], S. F. LACKOVIC[1], R. H. TOBE[3], P. W. GLIMCHER[2], E. A. PHELPS[1,3,2];
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Abstract: Many decisions involve weighing immediate gratification against future consequences. In such intertemporal choices, people often choose smaller, immediate rewards over larger delayed rewards. Although it has been suggested that emotion underlies impulsivity and the tendency to favor immediate rewards, we have previously shown that emotional arousal (specifically, pupil dilation responses), as well as choices, in intertemporal choice tasks are reference-dependent (Lempert et al., 2015). Arousal increases when less predictable rewards are better than expected, whether those rewards are immediate or delayed. Furthermore, when immediate rewards are more variable than delayed rewards (Immediate Vary condition), participants tend to be patient. When delayed rewards are more variable (Delay Vary condition), people are more impulsive. We tested whether emotional arousal causes this reference-dependence in intertemporal choice by pharmacologically blunting arousal responses using propranolol, a beta-adrenergic receptor antagonist. We administered propranolol and placebo in a two-day, double-blind within-subjects design, hypothesizing that propranolol would reduce the difference in impulsivity between the Immediate Vary and Delay Vary conditions in an intertemporal choice paradigm. Replicating our previous study, in the placebo condition, participants were significantly more impulsive in the Delay Vary than the Immediate Vary condition. This effect was weaker when individuals were given propranolol. This suggests that emotional arousal underlies sensitivity to the choice context during intertemporal choice.

Disclosures: K.M. Lempert: None. **S.F. Lackovic:** None. **R.H. Tobe:** None. **P.W. Glimcher:** None. **E.A. Phelps:** None.

Poster

720. Human Decision Making: Risk and Impulsivity

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Topic: F.01. Human Cognition and Behavior

Support: DFG Grant PE 167/3-1

Title: The role of reward immediacy in temporal discounting

Authors: *U. BROMBERG[1], C. BÜCHEL[2], J. PETERS[1];
[1]Dept. of Systemic Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Univ. Med. Ctr. Hamburg Eppendorf, Hamburg, Germany

Abstract: Temporal discounting choices which include an immediate reward (now-trials = NT), might give rise to more impulsive behavior than choices which do not (not-now-trials = NNT) (McClure et al., 2007). Exerting self-control during temporal discounting (i.e. choosing the larger later (LL) option) has been associated with the LPFC (Cohen & Lieberman, 2010; Hare et al., 2014, Figner et al., 2010). Here we investigate neuronal correlates of decision-making in relation to reward immediacy in a sample of young adults (n=81, age 18-20 years). We hypothesized that choice-dependent modulation of value-processing would be stronger during NT as compared to NNT, because the tempting option of an immediate reward in NT is expected to require stronger self-control than only delayed options in NNT (Figner et al., 2010). While we did find a main effect of lPFC-correlates when choosing the LL across all trials, contrary to our hypothesis, no neural correlates of choice where bigger in NT as compared to NNT. Furthermore the main effect of choice seemed to be driven by NNT, proving both dorsal and ventral PFC areas to be correlated with choosing the LL in NNT but not in NT. To further investigate the role of lPFC also in NT, we hypothesized that a stronger lPFC regulation during NT may only be required in high-discounting subjects, who are likely to be particularly tempted by the immediate reward option. Confirming this hypothesis only the high-discounters showed significant lPFC activation (both dorsal and ventral) also in the NT, when choosing the LL. While our results confirm a role of lPFC in self-control processes during discounting, they partly reveal a specific relevance for those who discount rewards more steeply. This result suggests an important role for individual differences in neural correlates in discounting behavior, in particular with respect to involvement of prefrontal regions implicated in self-control.

Disclosures: U. Bromberg: None. **C. Büchel:** None. **J. Peters:** None.

Poster

720. Human Decision Making: Risk and Impulsivity

Location: Hall A

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Program#/Poster#: 720.03/Z3

Topic: F.01. Human Cognition and Behavior

Title: Treating impulsivity: temporal discounting in heroin users undergoing treatment

Authors: *S. LOPEZ-GUZMAN[1], A. B. KONOVA[1], J. ROTROSEN[2], S. ROSS[3], P. W. GLIMCHER[1];
[1]Ctr. for Neural Sci., NYU, New York, NY; [2]Psychiatry, NYU Sch. of Med., New York Univ., New York, NY; [3]Div. of Alcoholism and Drug Abuse, Bellevue Hosp. Ctr., New York, NY

Abstract: Objective: Impulsive decision-making is a hallmark of addiction and, in the case of opioids like heroin, seems to be modified with treatment consisting of standard counseling and pharmacological maintenance therapy. Previous studies have used intertemporal choice procedures to measure the discount rate as a quantitative means for estimating impulsivity. However, whether discount rates can be used to predict and accurately track opioid use disorder patients' path through treatment remains a pressing open question. Its usefulness as a predictor of relapse or recovery is of critical importance. Opioid use is skyrocketing and opioid use disorder has one of the highest rates of relapse across substances of abuse. We conducted a longitudinal within-subjects study with repeated measures of temporal discount rates in a cohort of patients starting treatment for mild to severe opioid use disorder. Methods: Repeated measures (weekly and bi-weekly) of both an intertemporal choice task and a risk attitude task were made in a group of patients starting their treatment at Bellevue Hospital's Methadone Treatment Program. Both tasks were fully incentive compatible. A group of matched healthy controls performed the same tasks. The Time Line Follow Back questionnaire was used to track drug use between sessions, including use of patients' prescribed maintenance medication. Urine testing confirmed these reports. Subjects' choices in the behavioral tasks were fitted with a standard hyperbolic discount model and a second hyperbolic discount model, which incorporated a risk parameter to account for nonlinearities in the utility function. Results and conclusions: As previously reported, opioid use disorder patients have significantly higher discount rates compared to healthy controls. Preliminary results from our pilot study indicate discount rates follow each patient's clinical state through recovery, decreasing progressively with abstinence. Interestingly, discount rates also correlate with relapse events, peaking when these events occur. Data collection in a larger sample is ongoing. We conclude that temporal discounting, when assessed repeatedly over the course of treatment, could be used as a behavioral signature of patients' evolution and potentially serve as a useful predictor of prognosis and treatment adherence for opioid use disorder.

Disclosures: S. Lopez-Guzman: None. **A.B. Konova:** None. **J. Rotrosen:** None. **S. Ross:** None. **P.W. Glimcher:** None.

Poster

720. Human Decision Making: Risk and Impulsivity

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Topic: F.01. Human Cognition and Behavior

Support: DFG grant 1627/3-1

DFG grant 1627/5-1

Title: The interaction of episodic future thinking and temporal discounting in pathological gambling

Authors: *A. WIEHLER, J. PETERS;
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Abstract: Delayed rewards are devalued by human and non-human agents, a phenomenon know as temporal discounting (TD). Similar to many addiction disorders, pathological gambling (PG) is associated with steep devaluation of delayed rewards (Petry, 2001, J abnorm psychol). Recent studies reported that the ability to imagine future events (episodic future thinking, EFT) can interact with TD such that increased EFT leads to more foresighted decisions and attenuated discounting (Lin et al., 2014, Behav neurosci; Palombo et al., 2014, Hippocampus; Peters et al., 2010, Neuron). This interaction is of high clinical relevance for highly impulsive populations such as PGs or addiction patients in general. PGs do not show an overall impairment of EFT (Wiehler et al., 2015, submitted), but the interaction of EFT and TD and the neuronal underpinnings have not yet been studied in PG. We investigated a sample of n=24 pathological gamblers fulfilling the DSM-5 criteria and n=24 matched healthy controls (HCs) with fMRI during a TD task. The identical task was performed by n=6 (respectively n=7) behavioral pilots. The TD task consisted of two conditions: in the control condition, offers about delayed rewards were presented with a numerical amount and delay. In the episodic condition, rewards were presented in the same way, but enriched by participant-specific personal future event cues (based on a pre-scan interview, Peters et al., 2010, Neuron). We modeled participants decisions using the hyperbolic discounting model by Mazur (1989, J exp anal behav). k parameters (representing the degree of discounting) were significantly higher in the PGs than in healthy controls (main effect of group: $p < 0.01$). TD tended to be attenuated during the presence of episodic cues (main effect of condition: $p = 0.09$). This effect does not differ between groups (group x condition interaction: $p = 0.44$). On the neuronal level, both groups showed a robust activation of the EFT network (PCC, vmPFC) in the episodic condition (conjunction analysis: FWE corrected $p <$

0.05). We replicated previous findings of increased temporal discounting in pathological gamblers. However, similar to HCs, PGs tended to benefit from the presence of episodic cues by showing attenuated discounting. This finding might provide an interesting avenue for novel therapeutic interventions in pathological gambling and addiction more generally.

Disclosures: A. Wiehler: None. **J. Peters:** None.

Poster

720. Human Decision Making: Risk and Impulsivity

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Topic: F.01. Human Cognition and Behavior

Support: DOD ICB Contract W911NF-09-D-0001

Title: Tracking the neural dynamics of hypothesis evaluation with model-based fMRI

Authors: *N. MARINSEK, B. O. TURNER, M. B. MILLER;
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Abstract: In this study, we aimed to 1) model the component processes of hypothesis evaluation during the receipt of new evidence and 2) identify brain regions that support these processes. We used fMRI data from a previous experiment in which participants attempted to generate appropriate category labels for a series of word sets that were designed to either elicit repeated cycles of hypothesis formation and evaluation ("hard" word sets) or minimize these processes ("easy" word sets). We modeled participants' hypothesis certainty over the course of each trial as a Bayesian process and then conducted a model-based analysis of the fMRI data to identify brain regions with activity profiles that correspond to the predictions of our Bayesian model, such as absolute hypothesis certainty, changes in hypothesis certainty, and expectancy. We found that the left ventrolateral prefronal cortex (vlPFC) and left occipital cortex were more active when hypotheses were uncertain and the brain regions in the canonical default mode network (DMN) were more active when hypotheses were certain. These results are in line with previous research and suggest that the left vlPFC may play a role in forming hypotheses. We also found that activity in the left primary motor cortex increased when hypothesis certainty increased, even though we controlled for motor activity and motor preparation in the model. We propose that this activity reflects the motor preparation that results from discovering (or honing in on) the correct category. The results of this study provide insight into the psychological and neural processes of hypothesis evaluation, as well as the validity of Bayes' theorem as a model of belief updating in

humans. This research was supported by the Institute for Collaborative Biotechnologies under grant W911NF-09-D-0001.

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Poster

720. Human Decision Making: Risk and Impulsivity

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Support: Institute for Research on Gambling Disorders

John and Mary Franklin Foundation

Title: Neural manifestations of decision making are insensitive to confidence among pathological gamblers

Authors: *M. E. HUDGENS-HANEY[1], J. P. HAMM[2], E. A. KRUSEMARK[3], A. S. GOODIE[2], J. E. MCDOWELL[2], B. A. CLEMENTZ[2];
[1]Dept of Psychology, [2]Depts of Psychology & Neurosci., Univ. of Georgia, Athens, GA; [3]Dept. of Psychology, Univ. of Wisconsin, Madison, WI

Abstract: Pathological gambling disorder, classified in DSM-5 as an addictive disorder, is associated with severely maladaptive gambling behavior, often with dire economic and personal consequences. Pathological gamblers (PG) display both more confidence and more overconfidence on gambling tasks compared to frequent but non-pathological gamblers (NPG) and also fail to modulate bet acceptance according to their confidence level (Goodie, 2003). In addition, PG accept bets as if they have control over a betting situation even when the relevant control does not exist (Hudgens-Haney et al., 2013). Describing the neural circuits associated with confidence and control assessment, as well as aspects of this circuitry which are aberrant in PG, could lend critical information for understanding the neurophysiology of pathological gambling. In the current study, 34 PG and 34 NPG completed the Georgia Gambling Task, which begins with a standard confidence assessment task using 180 pairwise comparisons of US state populations. In a subsequent magnetoencephalography session, participants in the "Knowledge" condition viewed the same comparisons (Choice; 2000 ms), followed by the number of points that could be lost during trial (Cost; 1500 ms), and then accepted or rejected the bet that their previous answer was correct with a button press. After bet acceptance or rejection,

participants received feedback on whether they won or lost. The "Random" condition was similar in stimulus sequence except that, rather than state pairs, participants were presented matched uncontrollable probabilities of winning that bet on each trial. Oscillatory power was used to assess manifestations of stimulus and decision making processing at different frequencies. Main effects of gambling pathology, task control (Knowledge vs Random), and confidence level, as well as interactions, were found at various frequencies throughout the trial duration. Prior to the Choice, NPG showed a greater beta desynchronization than PG in occipital cortex. In addition, NPG showed greater beta activity in left visual regions during LC than HC, while PG did not modulate based on confidence level. A number of 3-way interactions were also found in bilateral visual regions. In response to the Choice, NPG, but not PG, modulate high gamma-band activity according to their confidence level, and only during the Random condition trials. These findings provide neurophysiological evidence that PG suffer from deviant confidence and control assessment and suggest that manifestations of top-down bias signals in early sensory processing are dysregulated in PG.

Disclosures: M.E. Hudgens-Haney: None. **J.P. Hamm:** None. **E.A. Krusemark:** None. **A.S. Goodie:** None. **J.E. McDowell:** None. **B.A. Clementz:** None.

Poster

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Support: EU Framework 6

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Title: Does temporal discounting predict future drug use? Structural mri and behavioral assessment at 14 and 18 years old

Authors: *S. MACKEY[1], B. CHAARANI[1], P. SPECHLER[1], K.-J. KAN[1], K. HUDSON[1], C. ORR[1], R. R. ALTHOFF[1], H. GARAVAN[1], T. HE IMAGEN CONSORTIUM[2];
[1]Psychiatry, Univ. of Vermont, Burlington, VT; [2]EU Framework 6, London, Dublin, Berlin, Paris, Germany

Abstract: Greater temporal discounting has been associated with a range of problematic impulsive behaviors including substance abuse, obesity, pathological gambling, as well as poor health and financial decision-making. Here, we examined the neural correlates of temporal discounting in a large group (N=1830) of 14 year old adolescents. Temporal discounting and drug use was assessed again at 18 years old to determine the stability of the measure across time and the relation of temporal discounting at age 14 to subsequent substance use. Methods Kirby's Monetary Choice Questionnaire (MCQ) was administered to a large sample of adolescents at 14 and again at 18 years old. The MCQ contains 27 items probing the subjects' preference for a range of small immediate rewards versus larger delayed rewards, e.g. item 5 asks: "Would you prefer €14 today, or €25 in 19 days?" Drug use history was assessed with the European School Survey Project on Alcohol and Drugs (ESPAD) questionnaire. T1-weighted magnetization prepared gradient echo sequence (MPRAGE) structural MR-images were obtained at age 14 years old and processed with the optimized voxel-based morphometry (VBM) method. Results The VBM analysis indicated that three cortical clusters (i.e. bilateral insula and frontomedial cortex) and one subcortical cluster including the anterior thalamus and ventral striatum were significantly related to temporal discounting, $p<0.05$ after correcting for multiple comparisons. Temporal discounting was positively correlated with the combined lifetime frequency of alcohol, nicotine, and illicit drug use ($r = 0.115$, $p<0.001$) and a composite measure of anti-social behavior ($r = 0.129$, $p<0.001$) at age 14. The overall rate of temporal discounting declined slightly from the age of 14 to 18 but the change was not statistically significant ($t(1312) = -1.65$, $p = 0.24$). The correlation between temporal discounting at 14 and 18 years old was $r = 0.29$, $p < 0.001$. Among the adolescents who had not been exposed to alcohol, nicotine, or illicit substance at age 14, individuals who endorsed greater temporal discounting had consumed more alcohol and drugs by the time they reached the age of 18 ($r = 0.146$, $p < 0.01$) Conclusions We found that temporal discounting is associated with a specific set of brain regions which have previously been associated with reward processing (e.g. the ventral striatum and frontomedial cortex) and the representation of the physiological state of the body (i.e. the insula). The stability of temporal discounting across time and its ability to predict future alcohol and drug use support the use of temporal discounting as a marker of addiction processes.

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Poster

720. Human Decision Making: Risk and Impulsivity

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Title: The genetic and neural correlates of risky decision making in young adults with antisocial substance disorder

Authors: *H. YARDLEY[1], M. DALWANI[2], J. SAKAI[2], S. MIKULICH-GILBERTSON[2], T. CROWLEY[2], M. MCQUEEN[1];
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Abstract: Background: Behavioral disinhibition (BD), an underlying vulnerability and a highly heritable trait, encapsulates separate behavioral manifestations including impulsivity, conduct disorder, substance dependence, and other externalizing behaviors. We aim to uncover the common pathways associated with this behavior by combining functional magnetic resonance imaging (fMRI) and analysis of genomic pathways to shed light upon the genetic and neural determinants of these behaviors. Methods: We recruited 43 young adults scoring high for BD and antisocial substance disorder, aged 24-32 years from the Colorado Longitudinal Twin Study, as well as 41 age matched controls. Functional scans were acquired using a risk-taking fMRI paradigm called the Colorado Balloon Game (CBG). A genome-wide association study (GWAS) pathway analysis of BD was conducted on a larger sample from which these subjects were recruited. We will then test for associations between brain activation during risky and cautious decisions in the BD subjects with the genetic summary score after adjusting for age and IQ. Results: Hi-BD subjects (n = 43, 21 males, mean age = 28.18 ± 1.53) in comparison with average BD subjects (n = 41, 19 males, mean age = 27.96 ± 1.71) made significantly higher risky button presses on the CBG (Hi-BD: mean 53.05 ± 15.78, Avg-BD: mean 46.39 ± 13.39, $p < 0.05$). We are awaiting results on BD vs. genetic score association and brain activation association with genetic score after adjusting for covariates. Conclusion: This study provides a unique opportunity to study individuals with high BD and understand if severity of BD is associated with genetic underpinnings, and to understand to what extent brain activation during risky and cautious decision-making is associated with their genetic profile. Such an analysis would be the first step to understand the contribution of genetics to biological brain vulnerability during decision making in BD individuals.

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Poster

720. Human Decision Making: Risk and Impulsivity

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 720.09/Z9

Topic: F.01. Human Cognition and Behavior

Support: NIMH training grant T32MH019524

Title: Do we need to treat risk? Attitudes toward risk and ambiguity in opioid addiction

Authors: *A. B. KONOVA[1], S. LOPEZ-GUZMAN[1], J. ROTROSEN[2], S. ROSS[2,3], P. W. GLIMCHER[1];
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Abstract: Objective: Drug addicted individuals are thought to be excessively reckless and risky, but the relevant factors that drive these behaviors are poorly understood. Economics provides a set of tools to quantify at least three factors that distinctly contribute to an individual's propensity for risk taking: their tolerance of known risk (technical risk attitude), their tolerance of ambiguous or unknown risk (ambiguity attitude), and the randomness in their decision process (stochasticity). Therefore, to more fully understand the behavior of opioid users in risky situations, in the present study we focused on these three factors, which have not been the subject of previous experimental decomposition in addiction - although such a decomposition may have important implications for understanding and potentially treating this disorder. Method: Individuals seeking treatment for an opioid use disorder at a large urban hospital completed a task in which they chose between a certain $5 and a lottery offering a chance to win more than $5 or nothing. Across trials, we varied the magnitude of the potential win, the probability of winning, and the level of ambiguity (how much was known about the probability of winning). The task was incentive compatible meaning that subjects were paid according to their actual choices. Results & Conclusions: Preliminary data suggest opioid users are more risk tolerant than they are ambiguity tolerant, as has been observed in health. We find good fits of a modified power utility model that treats ambiguity as a subtrahend term to probability, suggesting the assumptions of this model hold for the behavior of this group. Analyses that do not assume a specific model also support this claim: users are more likely to choose the lotteries when the amount and probability associated with these lotteries is higher, and the ambiguity lower. Data collection in an age and wealth matched community sample of non-drug users is ongoing. However comparison with parameters obtained in two independent published healthy adult data sets suggests opioid users may deviate more from health in their tolerance of ambiguity (~24-25% more tolerant) than of risk (~0-16% more tolerant). No apparent differences are observed for stochasticity, suggesting that a diminished "quality" of decision making is not driving these differences from health. These initial data raise the possibility that there may be ordering in the

relative contribution of these individual factors to the behavior seen in addicts (ambiguity attitude > technical risk attitude > stochasticity). Our ongoing work seeks to validate this hypothesis.

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Poster

720. Human Decision Making: Risk and Impulsivity

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DARPA SUBNETS

UC Irvine School of Medicine Bridge Fund

Title: Cortical activity underlying risk and reward decision-making

Authors: *I. SAEZ[1], J. J. LIN[2], E. CHANG[3], J. PARVIZI[4], G. SCHALK[5], P. BRUNNER[5], R. T. KNIGHT[1], M. HSU[1];
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Abstract: Generation of adaptive behavioral responses is not a passive process solely reliant on sensory evaluation. Rather, it requires active integration of sensory input with internally stored information such as generalized knowledge, previous experience, and goals. For example, variation in individual behavioral preferences, such as an individual's degree of aversion to risk, likely reflect underlying differences in internally generated neural activity. This active

combination of external information and internal activity is a fundamental, but understudied aspect of human decision-making. Brain oscillations have been proposed as a fundamental mechanism whereby activity within and across multiple brain areas is coordinated in the service of adaptive behavior. We recorded local field potentials from the brain of neurosurgical patients who underwent surgery for the treatment of intractable epilepsy using electrocorticography (ECoG) (n=7). ECoG signals reflect the coordinated activity of ensembles of hundreds of thousands of neurons, and are uniquely poised to reveal fast, circuit-level computations in the human brain. ECoG data was recorded from prefrontal cortical areas engaged in goal-oriented decision-making tasks, including orbitofrontal cortex (OFC) and lateral prefrontal cortex (LPFC) while patients engaged in a gambling game. We found that different aspects of the gambling game generated event-related changes in oscillatory activity across multiple areas and frequency bands. Specifically, we observed that in electrodes located in a variety of prefrontal locations there was a significant increase in high gamma (HG; 70-200Hz) power prior to choice in trials in which patients chose a safe prize over a gamble. A different set of electrodes showed differential HG activation at the time of gamble outcome reveal for behaviorally relevant aspects of the task (e.g. wins vs. losses), indicating that prefrontal regions carry out computations related to different aspects of the task. Cross-frequency coupling analyses revealed that the amplitude of HG was often related to the phase on slower oscillations on the theta frequency band (4-8Hz), suggesting a role for theta oscillations in coordinating local activity over multiple brain areas. These findings provide evidence that neural activity in lateral and orbital prefrontal regions may support cognitive processes underlying value-based decision-making such as cognitive control and reward-related learning, and highlight the role of ECoG recordings in advancing our understanding of the neural basis of risk and reward-related decision making in humans.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: KTIA NAP 13220150002 (DN)

János Bolyai Research Fellowship of the Hungarian Academy of Sciences (KJ)

Title: Individual differences in risk-taking: The role of executive functions and anxiety in predicting ambiguous events

Authors: *Á. TAKÁCS[1], A. KÓBOR[2], K. JANACSEK[4,3], F. HONBOLYGÓ[4,2], D. NEMETH[4,3];
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Abstract: Decision making is affected by several internal and external factors, including social effects, information availability, processing capacity, and personality traits. In our studies we aimed to investigate the contribution of individual differences in executive functions (EFs) and trait anxiety to the neural background of risky decision-making. The first study investigated the role of executive functions (EFs) in different strategies underlying risky decision making. Healthy adults participated in a Balloon Analogue Risk Task (BART) while ERPs were recorded. Participants were assigned to low EFs and high EFs groups based on their performance on a neuropsychological test battery measuring shifting, updating, and inhibition. In the experiment, each balloon pump was associated with either a reward or a balloon pop with unknown probability. The feedback-related negativity (FRN) associated with undesirable outcomes was larger in the high EFs group than in the low EFs group. As the FRN has been found to reflect salience prediction error, we suggest that the high EFs group formed internal models that were violated by the outcomes. The second study investigated whether nonclinical adults with high trait anxiety show smaller FRN for negative feedback than those with low trait anxiety in the BART. Participants were assigned to low and high trait anxiety groups by a median split on the State-Trait Anxiety Inventory trait score. Our results showed that the FRN for negative outcome was decreased in the high trait anxiety group compared to the low trait anxiety group. We propose that pessimistic expectations triggered by the ambiguity in the BART decreased outcome expectation errors in the high trait anxiety group indicated by the smaller FRN. In sum, our studies showed that expectations and prediction errors are modulated by EFs and trait anxiety at the neural level during risky decision-making.

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Poster

720. Human Decision Making: Risk and Impulsivity

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Program#/Poster#: 720.12/Z12

Topic: F.01. Human Cognition and Behavior

Title: Modulation of feedback-related negativity amplitude on the balloon analogue risk task

Authors: *A. W. MCCOY, M. E. YOUNG;
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Abstract: When making decisions, the probability and magnitude of errors can play a major role in changing preferences. Electroencephalography (EEG) research examining the error-related negativity (ERN) and the associated feedback-related negativity (FRN) has indicated that the amplitude of each component may predict subsequent behavioral change. The current study used a version of the Balloon Analogue Risk Task (BART) that involves outcomes that are dynamically changing over time. As the balloon grows, more points are available but the probability of the balloon popping (netting zero points) is higher; the participant decides when to stop the balloon's expansion to maximize points. The BART was adapted to facilitate the study of the FRN in dynamic environments. The purpose of Experiment 1 was to determine the effect of error magnitude on FRN amplitude during popped (incorrect) trials, whereas Experiment 2 was aimed at determining the effect of error magnitude on FRN amplitude during cashed-in (correct) trials. It was hypothesized that larger errors (i.e., the balloon popping after waiting a long time to cash-in) would result in a larger FRN than smaller errors. In Experiment 1, error magnitude did not contribute to the amplitude of the FRN. In Experiment 2, the masked points possible condition was a replication of Experiment 1. In the unmasked points possible condition, the number of points that could have been earned for each balloon was presented before participants found out how many points were earned. It was expected that there would be a larger FRN magnitude after cashed-in trials in the unmasked points possible condition compared to the masked points possible condition based on the magnitude of the error. In Experiment 2, the amplitude of the FRN was affected by the magnitude of the error on cashed-in trials in the unmasked condition, but not the masked condition. These results are seemingly at odds, and cannot be assimilated into any currently extant model of the FRN. An explanation relying on the motivational importance of errors is discussed.

Disclosures: A.W. McCoy: None. **M.E. Young:** None.

Poster

720. Human Decision Making: Risk and Impulsivity

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Support: JSPS KAKENHI 15H03062

JSPS KAKENHI 15K12363

Showa Women's University

Title: Performance and ERP in the Iowa Gambling task is related to body weight and daily eating behavior in Japanese healthy young females

Authors: F. ISHIKAWA, *K. YAMANAKA;
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Abstract: An impaired performance on Iowa gambling task (IGT) comes from decision-making that preferred a high immediate gain despite higher future losses. Previous studies demonstrated that obese individuals and patients with eating disorder, who are characterized by a preference for high immediate reward (excessive food intake) despite higher future punishment (weight gain), showed deficits on the IGT. Therefore, we hypothesized that, even in healthy participants, performance on IGT is correlated with their body weight, daily eating behavior, and ERP components related uncertain decision-making during the task. In this study, 10 Japanese healthy young female volunteers (height: 159.1±4.8, weight: 54.5±6.3, BMI: 21.5±2.1) conducted two sessions of the computerized IGT task, each containing 100 trials. In the IGT task, participants chooses from among four decks of cards, some of which yield higher immediate gain but larger future losses (disadvantageous decks) and others that yield lower immediate gain but smaller future losses (advantageous decks). They are asked to make choices that maximize their total gains. Task performance was assessed by (1) total number of choice for the advantageous decks minus total number of choice for the disadvantageous decks (net score), and (2) total number of choice for the largest loss cards (number of worst choice). Surface electroencephalography was recorded during the IGT task and analyzed by a traditional event-related potential (ERP) after the feedback onset. We addressed the relationships between a performance on IGT and body weight, daily eating behavior (Dutch Eating Behavior Questionnaire: DEBQ), and 2 ERP components, feedback-related negativity (FRN) and P300. Consequently, there was a significant negative correlation between body weight and net score. Also there was a significant positive correlation between body weight and number of worst choice. These results indicate that a participant whose weight is light can learn to make advantageous choices on the IGT. Next, amplitude of FRN in loss trials was negatively correlated with number of worst choice, while amplitude of P300 in loss trials was positively correlated with net score. Moreover, a participant whose score on restrained eating is high tended to be large FRN and P300 amplitudes. Overall results suggest that prefrontal function revealed by ERP components is related to not only IGT performance but also body weight and daily eating behavior even in healthy participants.

Disclosures: F. Ishikawa: None. **K. Yamanaka:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 721.01/Z14

Topic: F.01. Human Cognition and Behavior

Title: Moral transgressions and dirty bodies: Embodiment of the Macbeth effect is mapped topographically onto the somatosensory cortex

Authors: *M. SCHAEFER[1,2], M. ROTTE[2], H.-J. HEINZE[2], C. DENKE[3];
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Abstract: The theory of embodied cognition claims that cognitive representations are structured by metaphorical mappings from sensory experience. According to this theory knowledge is represented in modal systems derived from perception. Recent behavioral studies found evidence for this hypothesis, for example by linking moral purity with physical cleansing (the Macbeth effect). Neurophysiological approaches provided further support by showing an involvement of sensorimotor cortices for embodied metaphors. However, the exact role of this brain region for embodied cognitions remains to be cleared. Here we demonstrate that the involvement of the sensorimotor cortex for the embodied metaphor of moral-purity is somatotopically organized. Participants were asked to enact scenarios where they had to perform immoral (lying) or moral acts either with their mouths (leaving a message on a voice mail) or their hands (writing a note). Subsequently participants had to evaluate different products. Behavioral results showed that mouthwash products were particularly desirable after lying in a voice mail and hand wash products were particularly desirable after writing a lie, thus demonstrating that the moral-purity metaphor is specific to the sensorimotor modality involved in earlier immoral behavior. FMRI results of this interaction demonstrated associated activation in sensorimotor cortices during the evaluation phase that was somatotopically organized with respect to preceding lying on a voice mail (mouth-area) and preceding lying in a written note (hand-area). Thus, the results demonstrate a central role of the sensorimotor cortices for embodied metaphors.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: John Templeton Foundation / UC Berkeley / GGSC

Title: The effects of gratitude expression on neural activity and plasticity

Authors: *J. W. BROWN[1], P. KINI[1], S. MCINNIS[1], N. GABANA[1], J. WONG[2];
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Abstract: Gratitude is a common aspect of social interaction, yet relatively little is known about the neural bases of gratitude expression, nor how gratitude expression may lead to neural plasticity. To address these twin issues, we recruited subjects entering psychotherapy for depression and/or anxiety. One group was given psychotherapy plus a gratitude writing intervention, which required them to write a letter expressing gratitude. The control group was given psychotherapy only. After three months, subjects performed a "Pay it forward" task, similar to a trust game, in the fMRI scanner. In the task, subjects were repeatedly endowed with a monetary gift and then asked to pass it on to a charitable cause if they felt gratitude for the gift. We measured brain activity and found regions where activity correlated with self-reported gratitude during the task, even controlling for related constructs such as guilt motivation and desire to help. These were distinct from brain regions signaling empathy or theory of mind. We found other regions that correlated with trait gratitude measures. Finally, we found that a simple gratitude writing intervention led to significant neural plasticity - subjects who wrote a letter expressing gratitude showed significantly greater neural modulation by gratitude in the medial prefrontal cortex three months later.

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Poster

721. Social Cognition: Neural Processes and Disorders

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Program#/Poster#: 721.03/Z16

Topic: F.01. Human Cognition and Behavior

Title: Alteration of sensitivity toward the Holocaust related media content by negative Mass Media

Authors: *S. TUKAIEV[1], I. ZYMA[1], M. MAKARCHUK[1], N. PLAKHOTNYK[1], J. GRIMM[2], A. ENZMINGER[2], Y. HAVRYLETS[3], V. RIZUN[3];
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Abstract: Short-term effects of Mass Media are cumulative and lead to profound psychophysiological changes. It is well known that the negatively accented Mass Media causes insusceptibility to aggression and violence. The purpose of this study, a part of the project "Broadcasting History in the Transnational Space", was to identify the impact of TV news on the perception and processing of the emotional frames of a historical documentary. 38 healthy volunteers (21 women and 17 men) aged from 17 to 20 years, divided into two groups, participated in this study. The first group (23 volunteers) was demonstrated a video set comprised of 80 negative images, selected from the Holocaust documentary "Night and Fog" (1955, France), and 80 neutral images. The second group (15 volunteers) was presented emotional frames taken from TV news plots (150 images) in order to investigate the pre-stimulus modulations of perception and processing of the emotional frames of historical documentaries. During the exposure, event-related potentials (ERPs) were recorded. We analysed average signal amplitude of ERPs in the time intervals 40-80, 80-120, 120-220, 220-300, 300-400 and 400-700 ms after the onset of the exposure. At the end of the experiment the participants assessed each set of images on the scales of "relaxing - activating" and "unpleasant - pleasant". We noted that the emotional frames taken from TV news plots are relatively weak emotional stimuli compared to the emotional frames of historical documentary. The subjects exposed to the pre-stimulus evaluated the Holocaust pictures as more unpleasant and more activating than those without it. We demonstrated that the ERPs recorded during the presentation of the negative (historical) images had various amplitudes of oscillations due to the preliminary affective impact of TV news frames. The ERP amplitude of P300 in frontal zones was bigger for the group without the preliminary exposure to the emotional TV news frames due to the reduced sensitivity to the content of images. In this case, the historical images demand less attention and less emotional efforts for emotional evaluation of visual information, analysis, retrieval of information from memory and semantic processes, namely search for the meaning of the pictures (occipital P300 and LPP). Thus, short-term media effects include alterations of sensitivity toward the emotional content of visual information.

Disclosures: **S. Tukaiev:** None. **I. Zyma:** None. **M. Makarchuk:** None. **N. Plakhotnyk:** None. **J. Grimm:** None. **A. Enzminger:** None. **Y. Havrylets:** None. **V. Rizun:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Program#/Poster#: 721.04/Z17

Topic: F.01. Human Cognition and Behavior

Support: CIHR PDF

Title: An investigation of shared population coding for olfaction and social processing

Authors: *T. R. KOSCIK[1], W. A. CUNNINGHAM[2], A. K. ANDERSON[3];
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Abstract: Many key components of the human brain networks critical for social cognition are homologous to regions critical for social chemosensory evaluation of conspecifics in most mammals. Moreover, humans rely proportionally little on olfaction for acquiring social information compared to other sensory modalities, particularly vision. We propose that the neural circuitry in human olfactory brain regions have been assimilated by other sensory modalities and processes. We predict that chemosensory processes have been repurposed and extended to process multimodal social information (Koscik & Tranel, 2012). To address this prediction, we have conducted a Representational Similarity Analysis (RSA) of BOLD-fMRI data (Kriegeskorte, et al., 2008). Using RSA is particularly beneficial for our analysis as it allows us to compare across stimulus modalities (olfactory and visual) and to quantify the similarity of neural representations between stimulus types. While in the scanner, participants viewed a series of images of faces, foods (non-social, odor-related), and objects (non-social, non-odor-related) and smelled a set of different odors. We predicted that neural representations of social stimuli (e.g., face image pleasantness) would be more similar to representations of olfactory stimuli (e.g., odor pleasantness) than they would be to representations of non-social/odor-related stimuli (e.g., food image pleasantness) and non-social/non-odor-related stimuli (e.g., object image pleasantness). In addition, we predicted that the informational content of representations and their relationships across modalities would vary between neural regions. For example, the amygdala may be associated with the intensity or arousal of face and odor stimuli (consistent with Anderson, et al. 2003), but not food or object stimuli or other information about faces and foods such as valence. Preliminary results indicate several brain regions where facial and odor attributes share representations but food and object representations are dissimilar, including ACC, lateral OFC, and vmPFC. These results are consistent with notion that the neural algorithms originally evolved for social chemosensory perception have been repurposed to extract socially-meaningful information from non-olfactory, visual stimuli.

Disclosures: **T.R. Koscik:** None. **W.A. Cunningham:** None. **A.K. Anderson:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Topic: F.01. Human Cognition and Behavior

Title: Social pain changes pain sensitivity in people with anxiety state

Authors: *R. AKAGUCHI, M. OSUMI, S. MORIOKA;
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Abstract: [Purpose]Pain is deep-related to emotion. The more people feel the anxiety and fear, the more they feel the pain. In recent years, the research of "social pain" has attracted attention. It had been defined that the social pain increased physical pain sensitivity, and the brain activity underlying physical pain was similar to that one underlying the social pain. In present study, we aimed to make clear that be change more pain sensitivity by feeling social pain in people of anxiety state. [Methods]29 healthy people participated in this study. Only 15 people in all were recorded electroencephalography (EEG) in addition to behavioral data. 64-channel EEG System (ActiveTwo; BioSemi B.V.,) was used for the data acquisition (Sampling rate, 512Hz). Electrodes was placed according to the 10/20 system Participant was assessed psychological state by State-Trait Anxiety Inventory 2 (STAI-2). Thermal prove (UDH-105, UNIQUE MEDICAL) was attached to the center of the left inner forearm. Participant underwent 2 blocks pain stimulus baseline pain (before social pain) and final pain (after social pain) to investigate whether the changes of physical pain due to social pain. One block was carried out 30 times. Assessment of pain intensity (stimulation of constant temperature 48℃) was using Visual Analog Scale (VAS). In order to cause participants social pain, participants read the uniquely scenario. The scenario which superior humans than hero appeared caused participants emotion feeling such as envy, elation, longing, inferiority, hostility in the present study. The degree of social pain by the uniquely scenario was assessed score by VAS. [Results & Discussion] In behavior data, in participants with anxiety state (participants with high score of STAI-2 were 16 in all), the pain intensity had negative correlation with elation score, and elation score had negative correlation with the score of STAI-2. And Pain intensity increased significantly in final pain in participants with low elation score (participants with low score of VAS were 14 in all). In EEG data, increase of the amount of pain had negative correlation with the power spectral density of prefrontal cortex (alpha band 8-13Hz). These results suggest that participants with

anxiety state have no thought process, such as the solution to social stress factors. If there is no such thought process, activation of the prefrontal cortex increases by social stress, it led to an increase in pain. In other word, it considered that it is important for the control of pain to change thought that stress factor is one that grow their.

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Poster

721. Social Cognition: Neural Processes and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: KAKEN25700015

KAKEN22240026

Title: Social anxiety tendency affects event-related potential (ERP) during gaze perception

Authors: ***Y. TSUJI**[1], S. SHIMADA[2];
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Abstract: Information which we obtain from person's eye gaze is important in human social interaction and communication. We used event-related potential (ERP) to examine the effects of social anxiety tendencies on eye gaze perception. Avoidance or excessive fear is a defining feature of social anxiety disorder (SAD) or social phobia (SP) in a situation associated with being evaluated or embarrassed by others. Especially, gaze of others is known to frequently induce social anxiety. Sixteen healthy adult subjects (8 females, aged 21 ± 1.3, mean ± SD) participated in this study. Participant's level of social anxiety was examined by means of the Japanese version of the Liebowitz Social Anxiety Scale (LSAS-J). Subjects were divided into two groups, high social anxiety (HSA, n=9) and low social anxiety (LSA, n=7), on the basis of cut-off point which is at 60/144 in which SAD is probable. The experimental stimulus was either gray scale images of direct or averted eye gaze (leftward-gaze or rightward gaze), or closed eyes. In each trial, the experimental stimulus was displayed for 500ms, and then fixation cross appeared for 1500-2000ms (jittered). An experimental session consisted of 120 trials, and the subject underwent two experimental sessions. Electroencephalogram (EEG) signals were

recorded from 30 scalp sites, located according to the extended international 10/20 reference system. The amplitude and latency of P200 component of ERP at Fz were entered into two-way ANOVAs with conditions (direct, averted or closed) and groups (LSA or HSA). For amplitudes, there was a significant main effect of groups ($F(1, 14) = 9.3$, $p < .01$), which shows that amplitude of P200 was higher in HAS than in LSA. For latency, a main effect of conditions ($F(2,28) = 25$, $p < .05$) and an interaction between conditions and groups ($F(2,28) = 4.6$, $p < .05$) were significant. *Post hoc* analysis (Tukey's honestly significant difference; HSD) revealed that the latency was shorter for direct gaze and averted gaze than for closed eyes in LSA group ($p < .01$), while HSA group showed the shortest latency in the direct condition, followed in order by the averted and the closed conditions ($p < .05$). These results suggests that other's direct gaze is prominently processed in the subject who has higher tendency towards social anxiety.

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Poster

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Title: Mu rhythm desynchronization during the observation of emotional and non-emotional facial expressions in 30-month-old infants

Authors: *H. RAYSON[1], J. BONAIUTO[3], P. F. FERRARI[4], L. MURRAY[2];
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Abstract: Previous research has demonstrated overlapping neural activations for both observation and execution of facial expressions in adults. These shared activations have been assumed to provide indirect evidence for a human mirror neuron system (hMNS), which has been suggested to contribute to action recognition. The hMNS has been hypothesized to play an important role the development of infants' understanding of facial expressions; however, so far, no research has provided neural data to support this claim. The purpose of this study was to explore whether there is evidence of a functioning MNS in 30-month-old infants during observation of both emotional and non-emotional facial expressions. High-density EEG was used

to assess infant mu rhythm desynchronization, an index of MNS activity, while infants observed video clips of actors performing a number of facial expressions. The dynamic facial stimuli comprised four experimental conditions; a positive-emotion condition (joy), a negative-emotion condition (sadness), a non-emotional condition (mouth opening), and a control condition (scrambled facial movements). Any instances where infants imitated the facial expressions observed were also coded offline from video recordings made during stimulus presentation, in order to relate hMNS activity with imitative responses. Results revealed mu rhythm desynchronization in central motor areas for all experimental conditions compared to scrambled facial movements, which suggests that the facial hMNS is functional in 30-month-old infants. Additionally, we found hemispheric differences between emotional and non-emotional facial expressions, suggesting that the facial hMNS interacts with lateralized circuits for emotion processing. These results are consistent with adult facial MNS studies, and support a simulation account of facial expression processing.

Disclosures: **H. Rayson:** None. **J. Bonaiuto:** None. **P.F. Ferrari:** None. **L. Murray:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Program#/Poster#: 721.08/Z21

Topic: F.01. Human Cognition and Behavior

Title: Do different brain systems support the ability to recognize negative and non-negative emotions following traumatic brain injury?

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Abstract: Many adult individuals with traumatic brain injury (TBI) have been found to be impaired in facial affect recognition. In particular, TBI patients tend to mislabel negative emotions (e.g., anger, sadness), but perform similarly to normal healthy controls (NC) when asked to recognize non-negatively valenced expressions (e.g., happiness, surprise). Although recent studies have proposed that this finding is an artifact due to intrinsic features of non-negative emotions or task related factors, another account suggests that a separate neural system, more likely to be affected by TBI, supports the ability to recognize negative emotions. In order to examine the latter possibility, we administered a dynamic facial emotion recognition task to individuals with TBI (N=26) and NCs (N=15). Participants with TBI performed significantly worse than NCs when labeling negative emotions ($p<.001$), but there was no significant

between-groups difference for non-negative emotions ($p>.1$) and no significant within-group or across-groups correlation between scores for positive and negative emotions ($ps>.05$). Inspection of scores for participants with TBI revealed high within-group variability. As TBI is characterized by diffuse traumatic axonal injury, we used DTI (fractional anisotropy) to examine the existence of different brain systems supporting recognition of negative or non-negative emotions in the TBI sample. We examined whether specific patterns of axonal injury correlated with impairment with negative or non-negative facial affect recognition. In particular, given previous reports of the amygdala's role in recognizing negative emotions, we hypothesized that TBI group performance on negative emotion items would selectively correlate with integrity of the uncinate fasciculus, the white matter tract connecting the amygdala with the prefrontal cortex, which is important for overall emotion recognition. Indeed, we found a significant positive correlation between right uncinate fasciculus integrity and negative emotion recognition ($r=.63$, $p<.001$), but not non-negative emotion recognition ($r=-.45$, $p>.05$,). Our results support a role of specific white matter tracts damage to impairment of negative emotion recognition reported in TBI patients. This knowledge has the potential to serve a tool to predict (and target with ad hoc rehabilitation) TBI patients who will show emotion recognition deficits in the chronic phase. Future directions include enlarging the current sample and examining how functional connectivity at rest in different brain network supports negative and non-negative facial affects recognition.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: PRESTO sakigake (237602)

Kakenhi (22300139)

Title: Transcranial direct current stimulation to dorsolateral prefrontal cortex enhances the drop of activity in the ventral prefrontal social brain network during economic game

Authors: T. NIHONSUGI[1], *M. HARUNO[2];
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Abstract: Recent studies have shown that transcranial direct current stimulation (tDCS) to dorsolateral prefrontal cortex changes human economic behavior. However, little is known about how the tDCS stimulation acts on the whole network of the brain. To address this issue, we conducted functional magnetic resonance imaging (fMRI) experiment of a modified trust game (Nihonsugi, Ihara and Haruno), simultaneously using tDCS. This task was designed to dissociate the neural substrates for guilt aversion and inequity aversion, where player A chooses either 'in' or 'out' option. When player A chooses 'in', he also reveals an expectation probability of how likely player B will choose 'cooperate'. With the knowledge of the probability, player B then decides whether to 'cooperate' or 'defect'. We set inequity and guilt orthogonalized in the task. We asked subjects (n=22) to play a role of B twice in an MRI scanner (i.e., decide whether to cooperate or defect. One session was with tDCS and the other was the sham session, and their order was randomized across subjects. Behaviorally, tDCS stimulation to the right DLPFC increased guilt aversion ($p<0.05$), without affecting inequity aversion. This observation is highly consistent with our previous study (Nihonsugi, Ihara and Haruno). We next contrasted brain activity at the timing of game presentation (duration=reaction time) between tDCS and sham conditions, and then averaged across subjects (one-sample t-test, SPM12). We found a significant activity in the lateral (MNI: (-38 34 -10)) and medial (MNI: (-12 30 -8)) orbitofrontal cortices, and ACC (-10 46 34) by the sham - tDCS contrast ($p<0.001$; uncorrected). We also found larger drop of activity in these areas in the tDCS session. These results demonstrate that tDCS stimulation to DLPFC enhances the drop of activity in the ventral prefrontal social brain network, highlighting the importance of the interaction between DLPFC and the ventral social brain network in the realization of guilt aversion.

Disclosures: T. Nihonsugi: None. **M. Haruno:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Topic: F.01. Human Cognition and Behavior

Title: Neural specializations for interpersonal interaction in a competitive gambling task

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Abstract: Dynamic interpersonal interaction is integral to typical human social behavior. Although recent dyadic studies have investigated the paired neural correlates of communicative and cooperative tasks, little is known about the paired neural correlates that underlie competitive tasks requiring social interaction. Conventional neuroimaging techniques such as fMRI have identified key social regions in single brains specifically activated by competing with a human rather than a computer opponent, particularly the temporal-parietal junction (TPJ) [1]. A goal of our study is to extend these findings to interpersonal neural correlates between two brains in an ecologically valid context. We utilized near-infrared spectroscopy (NIRS) to directly measure BOLD signals simultaneously in pairs of subjects playing a simplified poker game. In this game, one player was first randomly dealt either a low or high card. This player was asked to bet or fold. The opposing player was then required to bet or fold in response to the action of the first player. The next trial then began with the opposite player receiving either a low or high card. This simplified poker game was played in two different conditions. In condition one, two subjects played the poker game against each other. In condition two, the same subjects simultaneously played against matched computer opponents. We hypothesized that neural areas potentially involved in social interaction, such as the TPJ, would be differentially sensitive to measures of interbrain coherence during the human vs human and human vs computer conditions. Wavelet analysis [2] was used to quantify coherence between the players for all regions sampled, including the TPJ. Behavioral results suggest that subjects play similarly, roughly obeying Nash equilibrium for a given set of contingencies regardless of the opponent. However, preliminary coherence findings indicate higher synchrony between cross-brain signals originating in the TPJ for the human vs human condition than for the human vs computer condition. These findings extend single brain studies of interpersonal competition to a dual brain paradigm and provide new evidence for a role of TPJ cross-brain coherence in social cognition.
1. Carter et al. (2012) A distinct role for the temporal-parietal junction in predicting socially guided decisions. Science, 337(6090), 109-111. 2. Torrence and Compo (1998) A practical guide to wavelet analysis. Bull of the Am Meteorological Soc, 79(1), 61-78.

Disclosures: M.R. Piva: None. **X. Zhang:** None. **A. Noah:** None. **S. Chang:** None. **J. Hirsch:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Topic: F.01. Human Cognition and Behavior

Title: Task-related activation and de-activation predict individual differences in both empathy and analytic reasoning

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Abstract: A large body of work has established that numerous attention-demanding tasks not only tend to activate a core set of brain regions (known as the task-positive network; TPN), but also tend to drive activity below resting baseline levels in a second large-scale brain network (known as the default-mode network; DMN). Some recent work also indicates that certain social tasks activate the default-mode network while de-activating the task-positive network. How does the balance of activity in these two networks reflect individual differences in performance and behavior? In this study, we examined the degree to which core regions of these two networks were activated and deactivated during social and mechanical tasks. We relate these patterns of neural activity to previously-established psychological measures of social and intellectual function. Following theoretical predictions from opposing domains theory (Jack et al. Neuroimage 2013), we predicted that high performance on measures of analytic reasoning would be associated with greater activation of the TPN and greater deactivation of the DMN during mechanical in comparison to social tasks. In contrast, we predicted that higher levels of empathetic concern would be associated with greater activation of the DMN and greater deactivation of the TPN during social in comparison to mechanical tasks. Finally, in line with the theoretical claim that activity of the DMN is more closely associated with empathetic concern than social skill, we predicted that performance on tasks measuring theory of mind would show neither pattern. Whole brain fMRI data was collected from college-aged participants who were presented with social and mechanical tasks. Following standard pre-processing, and selection of regions of interest from a statistically independently-determined mask, data were analyzed using a variety of statistical techniques including correlation, principal component analysis, and receiver operating characteristic (ROC) curves. The data strongly supported the first two hypotheses. Surprisingly, it was also found that theory of mind performance demonstrated a relationship with network activity that was more closely aligned to individual differences in analytic performance than in empathetic concern. These results suggest that examining the balance of activity in different networks can shed light on individual differences.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Grant-in-Aid for Scientific Research on Innovative areas (25119512)

Title: Estimation of inter-personal relationship in synchronous rhythmic communication by EEG and behavioral synchronizations

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Abstract: In our human communication, behavioral rhythms of different individuals is spontaneously synchronized through social interactions. Recent studies using electroencephalogram (EEG) have demonstrated the brain activities along with the behavioral performance in communication, that is to say, the subjects who are good at coordinating their behaviors with the other's behaviors showed low cognitive loads. However, it is not clear about the invisible inter-personal relationship between the subjects (e.g. leader or follower). In this study, we attempted to evaluate it with the causal analyses for the behavioral rhythms and EEG rhythms. We measured the behavioral rhythms and EEG from two subjects during an alternate tapping task where 2 subjects tapped a key alternately with their right finger. Subjects were required to tap the key with an equal time interval of previous tapping of the other subject, whereas the tapping rhythms were not instructed. We evaluated the ability of each subject by the possibilities of the presentation of synchronization with other tapping rhythms, that is, a difference between time intervals of previous other's tapping (from self to other) and current self tapping (from other to self). The behavioral results divided the subjects into good and bad pairs. Moreover, the causal analysis for the behavioral data showed the division of roles between subjects in pairs. Interestingly, in both good and bad pairs there are almost two types; either a static or a variable division of roles between subjects (static pair and variable pair). The static pairs clearly dissociated between the leader and followers, whereas the variable pairs alternately switched the roles. Furthermore, the time-frequency analyses for the EEG data showed the different frontal activities in the low frequency bands among the leader the follower in the static pairs and the variable pairs. These results suggested the possibility that the behavioral and EEG synchronization could estimate the inter-personal roles in human communication.

Disclosures: M. Kawasaki: None. **E. Miyauchi:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Program#/Poster#: 721.13/Z26

Topic: F.01. Human Cognition and Behavior

Support: JST CREST

Title: Neural substrates of motor coordination in joint action

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Abstract: In daily life, we have several kinds of interactions with others for performing joint action in which two or more persons integrate their actions for a common goal. Recent psychological and social studies have demonstrated that motor coordination in joint action could be affected by motor optimization and/or social interactions among the partners, although the underlying neural substrates are still unclear. Here, we report brain activities associated with a joint motor task. Nineteen dyads (38 participants) performed single and joint force production tasks in which the goals were to match their individual and averaged grip forces, respectively, to target forces (20% of their maximum grip forces) with minimum variability for 30 seconds. The dyads performed these tasks in a dual fMRI scanner system that allowed simultaneous recording of their brain activities during the tasks. Grip forces were recorded at 200 Hz using a non-magnetic grip force measurement system and were presented online along with the target force on a screen placed in the scanner room. The joint task required almost the same motor effort and had the same visual stimuli as the single task, but the force had to be controlled jointly by the dyads. Comparison of brain activities during single and joint tasks demonstrated that the joint task significantly enhanced activities around the medial prefrontal cortex, temporoparietal junction, and inferior frontal gyrus. This result suggests that brain activities for distinction and estimation of the self-other state play a crucial role in joint action.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: PRIN

Title: Social cognition in amyotrophic lateral sclerosis

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Abstract: Amyotropic Lateral Sclerosis (ALS) is a neurodegenerative disorder of upper and lower motor neurons. However, a high percentage of ALS patients show additional cognitive deficits involving executive functions. Recent evidence suggests that these deficits may extend to social cognition, impairing in particular the ability to correctly perceive and process the cognitive state of other people within a social contest. However, to date these observations have not been confirmed and tasks not specifically designed for adults have been used. The present study explored this issue in ten patients with ALS and mild cognitive deficits involving executive functions, using different experimental tasks. Thus patients were presented with a modified version of the Weapon task - a priming task used to test for race stereotype biases - and the Judgement Preference task - a task measuring Theory of Mind - in which the level of similarity with the actor was manipulated (in-group and out-group membership). Patients' performance differed from controls in both tasks. Specifically, while controls showed more classical speed advantage reflecting stereotype biases, this advantage was not present in controls. Furthermore, ALS patients were more impaired than controls in understanding the preferences of out-group than in-group members. These results suggest that subtle deficits in social cognition can be observed in ALS and might affect the ability of these patients to perceive and interact with conspecifics.

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Poster

721. Social Cognition: Neural Processes and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: Leverhulme

Title: Resting state functional connectivity of the anterior insula and inferior frontal gyrus predicts in-group bias

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Abstract: We examined whether biases in perception favoring in-group stimuli reflect learned changes in neural resting state activity (RSA). We examined differences in pre- and post-task RSA after football fans performed perceptual matching with in- or out-group associated stimuli. Functional connectivity was assessed from resting state fMRI scans based on three major networks in frontal cortex, the insula and parieto-occipital areas. Compared to pre-task activity,, post-task functional connectivity increased between the left AIC and the inferior frontal gyrus (IFG) and post-task connectivity was positively correlated with in-group bias in behavior. Post-task functional connectivity was significantly weaker between left anterior insula cortex (AIC) and dorsolateral prefrontal cortex (DLPFC), but this was unrelated to behavioral biases. These results suggest that functional connectivity between the AIC and IFG facilitates processing of the motivationally salient stimuli and this might explain the underlying mechanisms of enhanced performance in in-group perception.

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Poster

721. Social Cognition: Neural Processes and Disorders

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NSF IIP 1215327

Title: Modeling the neurodynamic interactions and organizations of teams

Authors: *R. STEVENS;
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Abstract: Across-brain neurodynamic synchronizations / organizations arise when teams perform coordinated tasks. Little is known about the frequency, magnitude, and duration of these organizations and their relationships with other performance variables. We present a symbolic electroencephalographic (EEG) approach that fills this gap by identifying when the neurodynamic organizations of teams occur and demonstrate its utility with three natural tasks, high school problem solving, submarine navigation, and perioperative teams. Each second, neurodynamic symbols (NS) were created showing the EEG power spectral densities (PSD) at the 1-40 Hz frequency bins for each team member. These data streams contained a history of the team's across-brain neurodynamic organizations, and the degree of organization was calculated from a moving average window of the Shannon entropy of task segments. Decreased NS entropy (i.e. greater neurodynamic organization) was prominent in the ~16 Hz EEG bins during problem solving, while during submarine navigation, the maximum NS entropy decreases were ~10 Hz and were associated with establishing the ship's location. In perioperative teams decreases in NS entropy were uniformly observed in both the 10 Hz and 16 Hz EEG bins. Neurodynamic synchronizations also occurred in the 20 - 40 Hz frequency PSD bins and were associated with other teamwork activities involving uncertainty or stress. The highest mutual information levels, calculated from the EEG values of team dyads, were associated with periods of decreased NS entropy, suggesting a link between these two measures. These studies show entropy and mutual information mapping of symbolic EEG data streams from teams can be useful for identifying organized across-brain team activation patterns associated with changing task activities and events.

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Poster

721. Social Cognition: Neural Processes and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: MOST 102-2420-H-004-009-MY2

MOST 104-2420-H-004-004-MY3

Title: Political preferences modulate neural correlates of trusting decisions

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Abstract: Trust is a key component that shapes inter-personal relationship and is known to vary with social contexts. Previous evidence has shown the power of ascribed identity (e.g., ethnicity, gender) upon trust behaviors of human beings. However, few studies have investigated the neural mechanisms underlying how acquired identity (e.g., political party) may influence one's trust-related decision making. To address this issue, we enrolled 58 healthy adults who share different political identities, defined by their presidential choices in 2012 Taiwan presidential election (i.e., KMT vs. DPP supporters), to participate in a repeated binary trust game experiment while undergoing fMRI scan. For each trial of the game, participants (investor) could choose to invest ("trust") their partner or not ("keep") in the first round and their partner would reply with either a "reciprocate" or "defect" feedback decision. Participants were informed that they would play the game with partners with the same, a different or no political identity. At the behavioral level, participants showed significantly higher probability of trust decisions when a partner shared the same political identity, suggesting that political identities indeed modulate their cooperative decisions. At the neural level, we found that identities defined by political preferences have different neurophysiological effects on decision outcomes. When playing with partners with the same political identity, functional contrasts between trials in which a partner defected participants' trust and trials in which a partner reciprocated participants' trust showed significant hemodynamic signal changes in brain regions including anterior insula (emotional processing), the temporoparietal junction (mentalizing), and the dorsolateral prefrontal cortex (self-regulatory control and/or working memory). In contrast, when playing with partners with a different political identity, participants exhibited greater activation in the striatum (reward learning) in response to trials in which a partner reciprocated as compared with trials in which a partner defected. More interestingly, increased activation in the anterior insula significantly correlated to closer perceived social distances between participants and their partners. In summary, these findings provide the first evidence on the neural foundations for the modulation effects of political identities upon trust behaviors, and indicate that studies of decision making should account for the role of social identity in altering behavior and brain response.

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Poster

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Title: Antisocial behavior and genetic variation in the oxytocin receptor

Authors: *D. HOVEY[1], M. LINDSTEDT[1], A. ZETTERGREN[1], L. JONSSON[1], A. JOHANSSON[3], J. MELKE[1], N. KEREKES[2], H. ANCKARSÄTER[2], P. LICHTENSTEIN[4], S. LUNDSTRÖM[2], L. WESTBERG[1];
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Abstract: Antisocial behavior is a major problem in all societies, and incurs significant public expenditures. The etiology of antisocial behavior is unclear, but it is well-established that genetic variation is a contributing factor. In light of indications that oxytocin may amplify prosocial behavior, dysregulation of oxytocin may consequently play a part in antisocial behavior. The aim of the current study was to investigate whether single nucleotide polymorphisms (SNPs) in the oxytocin receptor gene (OXTR) are associated with antisocial behavior. A discovery sample was drawn from the Child and Adolescent Twin Study of Sweden (CATSS; n=2372), and the Twin Study of Child and Adolescent Development (TCHAD; n=1232) was used for replication. The participants were assessed for aggressive and non-aggressive antisocial behavior, measured as continuous traits. Eight SNPs in OXTR were genotyped. Mixed model statistics were used for all statistical analyses. In the discovery sample, the rs7632287 AA genotype was strongly associated with higher frequency of overt aggression (directly targeting another individual) in boys, and this

was then replicated in the second sample. The C allele of rs4564970 was also associated with antisocial behavior in the discovery sample, but we were not able to replicate this in the second sample. We conclude that the rs7632287 SNP in OXTR may influence antisocial behavior in adolescent boys. Further replication of our results, as well as investigations into the underlying mechanisms, could be crucial to understanding how aberrant social behavior arises.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: The effects of prefrontal lesions and oxytocin receptor gene (OXTR) polymorphism on religious fundamentalism in traumatic brain injury (TBI) patients

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Abstract: The prefrontal cortex plays a critical role in regulating social beliefs, including religious beliefs. One important facet of religious beliefs is fundamentalism, which refers to the degree of belief adherence, and is related to intergroup bias. Given the important role of oxytocin in modulating social cognition including group processes, and past research identifying associations between polymorphisms of the oxytocin receptor gene (OXTR) with socio-emotional processes such as empathy and attachment style, it is likely that religious beliefs are similarly influenced by variations in OXTR. Genetic predispositions are critically involved in recovery and outcomes after brain injury, yet the impact of genetic factors and brain injury on religious beliefs has not been examined previously. In the current study, we investigated the effect of the OXTR genotype and brain lesions on religious fundamentalism in a large sample of patients with penetrating traumatic brain injury (pTBI). We genotyped the pTBI patients and

matched healthy controls for the OXTR rs53576 single nucleotide polymorphism (SNP), and measured their level of fundamentalism by administering the Religious Fundamentalism Scale. We found that lesion location and OXTR genotype interact to regulate degree of fundamentalism in patients with pTBI. In patients with lesions to the prefrontal cortex (PFC), the OXTR polymorphism did not affect level of fundamentalism; however, in patients with intact PFC, results revealed an effect of the OXTR polymorphism, with GG-allele carriers reporting higher fundamentalism than carriers of the AA or AG alleles. These findings show not only that lesion location and genetics interact to produce an effect on fundamentalism, but also that the integrity of PFC is necessary for the OXTR polymorphism to exert its influence. This result sheds light on the important role of OXTR genetic variation in mediating group affiliation processes and religious beliefs, which is also dependent on representations of social knowledge in the PFC.

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Poster

721. Social Cognition: Neural Processes and Disorders

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 721.20/Z33

Topic: F.01. Human Cognition and Behavior

Support: R01 MH090169

Title: Aberrant paralimbic network activity during the processing of moral violations in criminal psychopathy

Authors: *M. SIMMONITE[1], D. S. KOSSON[1], C. L. HARENSKI[2], V. D. CALHOUN[2], K. A. KIEHL[2];
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Abstract: A core feature of psychopathy is the commission of immoral acts against others. Prior imaging studies of the processing of moral stimuli have revealed psychopathy is associated with reduced limbic and paralimbic activity. However, no studies have investigated functional connectivity during the processing of immoral stimuli, and how activity within these networks relates to psychopathy. Incarcerated male inmates (n = 94) were rated for psychopathy using the Hare Psychopathy Checklist-Revised (PCL-R). Functional MRI data were collected across four sessions whilst participants were shown statements describing immoral, negative and neutral

acts. During the first two sessions participants were asked to indicate whether the statement describing a living thing (i.e., implicit condition). During the third and fourth sessions, participants were asked to judge whether or not the statement described an act that was 'wrong' or not (explicit condition). Group Independent Component Analysis (ICA) was used to decompose the data into 25 components. Component time courses were then regressed on to time courses describing the immoral, negative and neutral stimuli presented, with beta weights indicating the degree to which the component was modulated by the task. Consistent with hypotheses, components demonstrating significant correlations with psychopathy included a paralimbic network, for which beta weights for all three trial types correlated negatively with PCL-R scores during the implicit condition, but not the explicit condition. This suggests psychopathic traits are associated with abnormalities specific to certain aspects of moral processing. These results provide evidence for an association between aberrant functional connectivity and psychopathy, and potentially elucidate the neural underpinnings of subtle abnormalities of moral insensitivity in psychopathy.

Disclosures: M. Simmonite: None. **D.S. Kosson:** None. **C.L. Harenski:** None. **V.D. Calhoun:** None. **K.A. Kiehl:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01MH087525

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John Templeton Foundation

Title: Electrophysiological markers of distinct facets of empathy and their relation to trait empathy and psychopathy

Authors: *K. L. LEWIS, J. M. COWELL, J. DECETY;
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Abstract: Empathy can promote a variety of positive social behaviors, motivate us to help others, and lead to greater competency in social interactions. This construct can be divided into distinct components, and the present study sought to dissociate two of these components,

affective sharing and empathic concern, using high-density electroencephalography (EEG). A second aim was to relate the neural dynamic markers of affective sharing and empathic concern to individual differences in trait empathy and psychopathy. The experiment used a novel paradigm wherein participants viewed a validated set of painful and neutral stimuli, and had to focus on and rate either their feelings of concern for the individual or how much physical distress the individual was in (affective sharing). Both the early ERP component (175-275 ms) and the late positive potential (LPP; 400-1000 ms) showed differentiations of painful versus neutral stimuli, with painful stimuli eliciting larger ERP responses, and an effect of condition, with amplitudes greater for affective sharing than for empathic concern. Differences in painful versus neutral stimuli in the LPP, a more controlled response indexing elaborative processing of valenced stimuli, were related to individual differences in empathy and psychopathy, but no such differences were found in the early window. Interestingly, while there were no significant relations between the LPP to painful over neutral stimuli during affective sharing, the LPP during empathic concern was positively predicted by trait empathy and inversely associated with dispositional psychopathy. Additionally, gamma coherence, an index of multisensory integration that has been associated with understanding the subjective states of others, did not show task-based differences, but was related to dispositional empathic concern and negatively associated with trait psychopathy. As a whole, these results suggest that affective sharing and empathic concern can be distinguished at the neural level with high-density EEG/ERP, and that their electrophysiological signatures are differentially modulated by individual dispositional measures, lending support to the notion that they are separable constructs.

Disclosures: **K.L. Lewis:** None. **J.M. Cowell:** None. **J. Decety:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: NRF-2012R1A2A2A04047239

NRF-2013S1A3A2053282

Title: How do narcissistic people view others' minds?: FMRI studies on empathy and perspective-taking

Authors: *N. KIM[1], M. KIM[2], H. JUNG[1], S. KANG[2], J. KWAN[3], M. CHUNG[2], H. KIM[1]; [1]Psychology, Korea Univ., Seoul, Korea, Republic of; [2]Gachon Univ., Sungnam, Korea, Republic of; [3]Sung Shin Women's Univ., Seoul, Korea, Republic of

Abstract: Narcissism, defined as an unusually high affection towards oneself, can be primarily characterized by two key features: a lack of empathy for other people and self-centered perspective. Although these features are the signifiers in the diagnosis of narcissistic personality disorder for clinicians and are highlighted in lay public's impression of narcissistic people, few studies empirically investigated biological evidence supporting these core features of narcissism. As a result, the present understanding of its neural correlates remains poorly understood. In this study, we aimed to discover the neural processes of empathy and perspective-taking differentially modulated by the degree of narcissism. We hypothesized that high narcissistic individuals would show reduced empathic responses and/or perspective-taking, accompanied by neural processes distinguished from those of low narcissistic individuals. 38 non-clinical female participants with different degrees of narcissism score (Narcissism Personality Inventory-40) participated in these fMRI experiments. Subjects performed context-dependent facial emotion reading task (i.e., empathy task) and preference estimation task (i.e., perspective-taking task) while being scanned in MRI. In the empathy task, high narcissistic participants showed reduced emotional interpretations on contextually modulated ambiguous facial expressions. In addition, high narcissistic individuals also showed reduced bilateral insula activities, a brain region known to be involved in empathy processing, in response to negatively-cued facial expressions during empathy task. In the perspective-taking task, high narcissistic individuals had less correct guesses on estimating others' preferences with decreased activity in the dorsomedial prefrontal cortex (dmPFC), which had a positive relationship with accuracy during the estimation of others' preferences (Kang, Lee, et al., 2013). Furthermore, they also showed increased activity in the ventromedial prefrontal cortex (vmPFC), implicating an intuitive self-oriented valuation, while estimating the preferences of others vs. self. In sum, the present findings provide neural markers indicating impaired empathy and perspective-taking prevalently associated with narcissism.

Disclosures: **N. Kim:** None. **M. Kim:** None. **H. Jung:** None. **S. Kang:** None. **J. Kwan:** None. **M. Chung:** None. **H. Kim:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Program#/Poster#: 721.23/Z36

Topic: F.01. Human Cognition and Behavior

Support: CONACyT GRANT 138663

CONACyT GRANT 129303

Title: Decreased neuronal population and increase gliosis in Orbito Frontal, Dorso Lateral and Ventro Medial cortex of people who committed suicide

Authors: *E. BALTAZAR-GAYTAN[1,2], P. AGUILAR ALONSO[3], F. GARCIA DOLORES[4], A. DÍAZ FONSECA[5], R. VAZQUEZ ROQUE[6], F. DE LA CRUZ LOPEZ[7], G. FLORES[6]; [1]Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; [2]Posgrado en Ciencias Químicas, Área de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas, Univ. Autónoma de Puebla, Puebla, Mexico; [3]Área de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas, Univ. Autónoma de Puebla., Puebla, Mexico; [4]Inst. de Ciencias Forenses, Tribunal Superior del Distrito Federal, Av. Niños Héroes 132, Col. Doctores, C.P. 0627, MEXICO DF, Mexico; [5]Facultad de Ciencias Químicas, Benemerita Univ. Autonoma de Puebla,, PUEBLA, Mexico; [6]Lab. Neuropsiquiatria, Inst. de Fisiología. Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; [7]Lab. Fisiología de la Conducta. Escuela Nacional de Ciencias Biológicas, MEXICO DF, Mexico

Abstract: Suicide represents 1.8% of deaths in young people worldwide. Although many of them occur in people suffering from mood disorder, only a small number of these patients attempt suicide and even fewer commit suicide, suggesting that other factors, such as the neurobiological ones, play an important role in the suicide. Postmortem Neurobiological techniques of high-resolution images have shown brain morphological changes, especially in areas of the limbic system that are closely interconnected such as the medial prefrontal cortex (CMP). Changes regarding social behavior, decision-making and the emotional process are presented in patients with lesions in this region. When damage is produced to the central nervous system (CNS), the innate immune response activates glial cells (astrocytes and microglia) by synthesizing cytokines, prostaglandins and reactive oxygen species, this is a regulated process, nevertheless, if an excessive inflammatory response occurs it can damage cellular integrity, because CNS is vulnerable to uncontrolled immune and inflammatory processes. Currently there are no studies which reveal the density of neurons in prefrontal cortex of the brain in people who committed suicide; this paper shows the result of the analysis of CMP of several 14 to 24 years old suicide victims from Mexico City. They were evaluated using astrocitosis immunohistochemical markers such as GFAP and protein nitration as well as stereology to measure neuronal density. The results show that cell density is reduced and there is an increased in GFAP immunoreactivity as well as protein nitration in Orbito Frontal, Dorso Lateral and Ventro Medial Cortex . This implies that oxidative processes presented in the brains of suicide victims contributes to neurodegeneration, which consequently causes a reduction in the PFC cell number and also causes a reactive astrogliosis, which has been linked to the development of a chronic inflammatory response that contributes to neuronal death. On the other hand, it has been reported that the inflammatory process induces the expression of inducible nitric oxide synthase,

an isoform responsible for promoting the synthesis of nitric oxide, which in high concentrations promotes radical peroxynitrite formation. It is a highly oxidizing agent, capable of causing the nitration of amino acid residues of proteins. Consequently it generates a change in the structure and function of these proteins, which triggers the neuronal death mechanism.

Disclosures: E. Baltazar-Gaytan: None. **P. Aguilar alonso:** None. **F. Garcia dolores:** None. **A. Díaz fonseca:** None. **R. Vazquez roque:** None. **F. De la cruz lopez:** None. **G. Flores:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 721.24/Z37

Topic: F.01. Human Cognition and Behavior

Support: UL1TR000041

KL2TR000089

Title: Over-arousal as a mechanism between alcohol use and intimate partner violence

Authors: *B. C. FINK[1], E. CLAUS[3], J. F. CAVANAGH[2], D. A. HAMILTON[2], D. BARTO[2]; [1]Dept. of Psychiatry and Behavioral Sci., [2]Psychology, Univ. of New Mexico, Albuquerque, NM; [3]Mind Res. Network, Albuquerque, NM

Abstract: Intimate partner violence (IPV) is a significant public health problem for which there are currently no effective treatments. Alcohol use is present in most instances of IPV and is associated with an increase in the frequency and severity of IPV. We believe that alcohol may be related to the increase in frequency and severity of IPV through a process of over-arousal that results from the cortically and psychophysiological arousing effects of alcohol during the ascending limb of intoxication and at peak BAC compounded by the unique behavioral and affective patterns of violent couples. The first aim of the study is to determine if increases in arousal after alcohol exposure is potentiated by evocative partner stimuli and is greater for distressed violent (DV) partners than distressed nonviolent (DNV) partners. A second aim is to determine if alcohol induced arousal interfered with DV partners' ability to regulate emotion in response to evocative partner stimuli compared to DNV partners. The study is an experimental comparison of the effects of alcohol on arousal and emotion regulation between DV and DNV partners. To test the overall hypothesis that over-arousal is a mechanism through which alcohol

is associated with increases in the frequency and severity of IPV, the selected partners participate in a counter-balanced placebo session and an alcohol administration session during which electroencephalography (EEG), psychophysiology and pupillary response measurements of arousal are collected during an emotion regulation task. The data is analyzed using a repeated measures ANOVA with a between-subjects factor. DV partners experience significantly greater neurophysiological arousal than DNV partners during the evocative stimuli condition. DV partners also experience greater difficulty regulating emotion during evocative stimuli than DNV partners and that this effect was compounded during the alcohol administration condition. Findings from this study provide firm evidence that alcohol is associated with IPV through a mechanism of over-arousal and provide key targets for intervention to prevent future IPV.

Disclosures: **B.C. Fink:** None. **E. Claus:** None. **J.F. Cavanagh:** None. **D.A. Hamilton:** None. **D. Barto:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 721.25/Z38

Topic: F.01. Human Cognition and Behavior

Title: Psychophysiological correlates of impulsive aggression in real-time

Authors: *J. FANNING[1,2], R. LEE[2], M. BERMAN[3], E. COCCARO[2];
[2]Dept. of Psychiatry and Behavioral Neurosci., [1]Univ. of Chicago, Chicago, IL; [3]Dept. of Psychology, Mississippi State Univ., Starkeville, MS

Abstract: Chronic impulsive aggression has been linked to abnormal structure and function of brain regions supporting affective processing, cognitive control, and social cognition. However, very little research in humans has focused on the neural processes that underlie an aggressive interaction. Thirteen men and women completed a standard paradigm that simulates an aggressive interaction in a controlled laboratory setting. Participants interacted with a fictitious opponent with whom they exchanged shocks at varying intensity levels under the guise of competing in a reaction-time task. High-density electroencephalogram (hdEEG) was recorded during the social interaction. Event-related potentials (ERPs) and source localization were used to assess activity related to (1) the participant's evaluation of the opponent's provocative behavior, and (2) the participant's decision to retaliate against the opponent. ERPs showed that participants engaged in greater evaluation of provocative behavior by the opponent compared to less provocative behavior, based on peak P3 amplitude. However, the P3 amplitude was

unrelated to the participant's decision to escalate, de-escalate, or match in response to the opponent. Peak P3 latency was longer on trials in which participants escalated aggressively against the opponent compared to when they equaled the opponent's level of provocation or de-escalated, suggesting that the timing of stimulus evaluation may affect the expression of aggressive responding. Reaction times did not differ as a function of the participant's choice to retaliate. Source analyses (CLARA) conducted at P3 peak latencies reveal sources in the orbitofrontal cortex (OFC) and amygdala, among other regions. EEG provides a window into neural events as they occur in real-time. The current results suggest that intense provocation, a common precipitant to aggressive behavior, elicits greater recruitment of neural resources for stimulus evaluation. Extent of stimulus evaluation as indexed by P3 amplitude was unrelated to the decision to retaliate; however, the timing of stimulus evaluation was related to the participant's behavior in response to the opponent's provocation. Future work will be informative in identifying the neural processes that relate to abnormal aggressive responding to threat.

Disclosures: **J. Fanning:** None. **R. Lee:** None. **M. Berman:** None. **E. Coccaro:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Title: Attention deficit hyperactivity disorder: increased coupling in the salience network

Authors: *E. T. ROLLS[1], X. JI[2], T. GE[2], W. CHENG[2], J. ZHANG[2], L. SUN[3], Y. WANG[3], J. FENG[4];
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Abstract: Attention-deficit hyperactivity disorder (ADHD) is a common and impairing neuropsychiatric disorder with preschool onset that may continue into adulthood. We analyzed differences in the resting state functional connectivity of brain areas in 249 ADHD patients and 253 typically developing control children with the aim of advancing the understanding of the neural bases of the disorder, and using data from the ADHD-200 Consortium. A method termed a brain-wide association study (BWAS), was used which allowed an unbiased search of the functional connectivities between all pairs of 90 regions parcellated according to the automated anatomical labeling (AAL) atlas, and associated the differences in functional connectivities with the disorder. Significantly altered functional connectivity (p~10-5) found in the ADHD children was the increased coupling in the salience network, comprising the anterior cingulate cortex and the anterior insular cortex. Correlations of this altered functional connectivity were found with the ADHD score index (Conners' Parent Rating Scale-Revised, Long version), and with the inattentive and hyperimpulsive ADHD subscores. The voxel-level locations of the voxels with the most increased functional connectivity were [-12, 44, 10] (MNI X,Y,Z), which is pregenual cingulate cortex, and [-24, 28, 6] which is anterior insular cortex, though the insular voxels spanned Y=32 to Y=0). The insular part of the network is in a region involved in visceral-autonomic function (Rolls 2014), which functions differently in people with ADHD. This is the first evidence from a large and brain-wide study for the significant involvement of the salience network in ADHD. Rolls,E.T. (2014) Emotion and Decision-Making Explained. Oxford University Press: Oxford.

Disclosures: E.T. Rolls: None. **X. Ji:** None. **T. Ge:** None. **W. Cheng:** None. **J. Zhang:** None. **L. Sun:** None. **Y. Wang:** None. **J. Feng:** None.

Poster

721. Social Cognition: Neural Processes and Disorders

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 721.27/Z40

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant BCS-1344285

Title: Alpha power modulations predict student distractibility in a classroom setting

Authors: *L. WAN[1], S. DIKKER[2,3], I. DAVIDESCO[2], L. KAGGEN[2], J. ROWLAND[2], J. MCCLINTOCK[4], D. POEPPEL[2,5], M. DING[1];
[1]Univ. of Florida, Gainesville, FL; [2]New York Univ., New York, NY; [3]Utrecht Univ., Utrecht, Netherlands; [4]Trevor Day Sch., New York, NY; [5]Max-Planck-Institute, Frankfurt, Germany

Abstract: Alpha oscillations (8 to 12 Hz) are a prominent EEG phenomenon in humans. Goal-oriented modulation of alpha has been extensively studied. We report a case of alpha modulation by social context and show that it can be used as a biomarker to predict student distractibility in the classroom. Wireless EEG was recorded from students in a New York City high school biology class, both before and during 50-minute class periods. Before every class period, students (1) sat face-to-face and made eye contact with one another and (2) turned toward the wall and looked at a fixation point on the wall; these two resting-state conditions were used for evaluating the modulation of alpha power in each student. Pre- and post-class questionnaires were also collected to assess the physical and emotional conditions of the students and their class performance. After preprocessing, spectral analyses were applied to the EEG data, and alpha power was computed for the two resting-state conditions. We found that (1) alpha power decreased from the face-to-wall to the face-to-face condition, and this decrease was most pronounced in occipital and frontal regions, (2) there was a positive correlation between the students' distractibility and their alpha decrease, and (3) alpha decrease was negatively correlated with pre-class expectation and post-class satisfaction. There is also evidence that alpha power decrease was positively correlated with students' social group affinity. We interpret these results in the framework of distraction conflict model and in terms of the alpha's role in attention deficit disorder.

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Poster

722. Mechanisms of Attention II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 722.01/Z41

Topic: F.02. Animal Cognition and Behavior

Support: FWO

IUAP

Odysseus

Title: Task-related functional connectivity study of attentional control in monkeys

Authors: ***P. F. BALAN**, A. GERITS, W. VANDUFFEL;
KU Leuven, Leuven, Belgium

Abstract: We aimed to determine differences in top-down and bottom-up control of attention using monkey fMRI and psychophysiological interaction (*PPI*) *analyses*. Three macaque monkeys were trained to covertly detect a random (0.6 probability) dimming occurring with equal probability at one out of four locations at 6 deg. eccentricity in each quadrant (all objects 0.5 x 0.5 deg.). The target-dimming was cued either in a bottom-up or top-down manner, using a change in color of the target itself or the fixation point (symbolic cue for each quadrant), respectively. Target but not distractor dimmings (appearing at the 3 other quadrants) had to be indicated with a manual response. The monkeys were scanned using an event-related paradigm on a 3 T Siemens Trio scanner and an 8-channel receive coil (contrast-agent-enhanced (Vanduffel et al., 2001), 1.25 mm isotropic voxels). We measured cue related fMRI activations using standard GLM and task-specific network interactions using PPI. In addition, based on the beta-strength of the PPI interactions, we assessed network metrics (node/edge betweenness centrality; node strength) (Rubinov and Sporns, 2010) to characterize the topology of functional connectivity networks. The behavioral performance of all monkeys was better for bottom-up compared to the randomly-interleaved top-down cued trials (higher percent correct; shorter reaction times). While the GLM-defined activation patterns revealed only very marginal functional differences between key nodes within the parieto-frontal attentional network, task-based functional connectivity analyses showed a stronger contribution of LIP during bottom-up and FEF during top-down trials. In conclusion, subtle functional differences across nodes participating in attentional control might be easier to detect using task-based functional connectivity analyses compared to traditional GLM analyses. Put otherwise, it is not necessarily the degree of fMRI activation in the individual nodes but rather the interaction across nodes within a functional network which determines their functionality in attentional control.

Disclosures: **P.F. Balan:** None. **A. Gerits:** None. **W. Vanduffel:** None.

Poster

722. Mechanisms of Attention II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01EY005911

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NIH Grant F31MH103895

Title: Distinct neurobiological mechanisms of top-down attention

Authors: *T. Z. LUO[1], J. H. R. MAUNSELL[2];
[2]Neurobio., [1]The Univ. of Chicago, Chicago, IL

Abstract: Neuronal signals related to visual attention are found in widespread brain regions, and these signals are generally assumed to participate in a common mechanism of attention. However, the behavioral effects of attention in detection can be separated into two distinct components: spatially selective shifts in either the criterion or sensitivity of the subject. Here we show that a paradigm used by many single-neuron studies of attention conflates behavioral changes in the subject's criterion and sensitivity. Then, we designed a task to dissociate these two components and achieved consistent isolation of spatially specific behavioral changes. We then recorded from populations of single neurons in area V4 of visual cortex in monkeys performing the dissociation task. Multiple aspects of attention-related neuronal modulations in V4--increase in firing rate, reduction in noise correlation, and decrease in Fano factor--corresponded to behavioral shifts in sensitivity but not criterion. This result suggests that separate components of attention are associated with signals in different brain regions, and that attention is not a unitary process in the brain but instead consists of distinct neurobiological mechanisms.

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Poster

722. Mechanisms of Attention II

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Program#/Poster#: 722.03/Z43

Topic: F.02. Animal Cognition and Behavior

Title: Asymmetrical light experience affects endogenously determined lateralization pattern in the pigeon

Authors: *S. LETZNER, M. MANNS, E. UNVER, O. GUNTURKUN;
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Abstract: Cerebral asymmetries are a fundamental organization principle of the vertebrate brain. A model system to analyze the development and the influence of envirotypic factors onto cerebral asymmetries is the visual system of birds like pigeons and chickens. Asymmetrical light exposure of the bird embryo is responsible for the lateralization of visually guided behavior. Due

to the asymmetrical position of the embryo in the egg, the right eye/ left hemisphere is stimulated with light meanwhile the left eye/ right hemisphere is naturally light deprived. This induces functional asymmetries of the left hemisphere for discrimination processes. In contrast, the influence of light for right-hemispheric dominances like spatial attention processes is still unknown but necessary for a better understanding of the interrelations between the development of left- and right-hemispheric lateralization. In chickens, right-hemispheric lateralization for spatial attention processes vanishes after incubation of the eggs in complete darkness, indicating that the emergence of right-hemispheric specialization depends on asymmetric light stimulation as well. Since chickens and pigeons display similar light-dependent development, it is conceivable that right-hemispheric dominance for visuospatial attention also develops in response to asymmetrical visual stimulation in pigeons. Accordingly, also the critical interaction of left-and right hemispheric asymmetries can be similar to that in chicks. On the other hand, structural asymmetries differ between two different bird species, suggesting different developmental mechanisms of left- right-asymmetries and its reciprocal interactions. Therefore, we compared the lateralization pattern of pigeons with different embryonic light experiences (light and dark incubated). The birds were required to explore an area in front of them and to sample grains of food. The results showed that light incubated pigeons attended symmetrically to target stimuli located in the left and right hemispace, whereas dark incubated pigeons showed a tendency for the right hemispace. These results imply the presence of an endogenous left-hemispheric lateralization of visual attention processes that is modified by asymmetric ontogenetic light experience. Furthermore, this result hints to species-specific differences in the lateralization pattern of specific functions.

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Poster

722. Mechanisms of Attention II

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Topic: F.02. Animal Cognition and Behavior

Support: Y51HN41171

Y33FN21171

Title: Neural correlates of posterior parietal cortex in a cross-modal selective attention task

Authors: *Y. ZUO[1], Z. WANG[2];
[2]Chinese Acad. of Sci., [1]Inst. of Neurosci., Shanghai, China

Abstract: In daily life, humans have to consistently process and integrate information of stimuli in different sensory modalities simultaneously. Voluntary 'top-down' attention is a key mechanism to select relevant subsets of sensory information for detailed and effective processing, and to actively suppress distracting irrelevant sensory information. The posterior parietal cortex (PPC) has been implicated to play a role in shifting attention from one perceptual dimension of a stimulus to another. This study examined how PPC is involved in attention shifting from one modality to another. We trained rats to perform a bimodal attention shift task. In this task, subjects have to selectively attend to a stimulus in one modality and respond to it, whereas the stimulus in the other modality has to be ignored. The subjects must alternate which modality they select multiple times within each session. Neurons in PPC were recorded during this task. We found that PPC neurons showed similar response pattern to the attended stimulus either when it was presented alone or in combination with a distractor, whereas response to ignored distractor was inhibited. These results suggest that PPC plays an important role in modality gating.

Disclosures: Y. Zuo: None. **Z. wang:** None.

Poster

722. Mechanisms of Attention II

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Program#/Poster#: 722.05/AA1

Topic: F.02. Animal Cognition and Behavior

Support: Fondecyt 1100465

Title: The activation of the lateral septum increased the release of brain histamine

Authors: *P. FARIAS[1], J. DIAZ[2], A. OCAMPO[2], J. VALDES[2], F. TORREALBA[3];
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Abstract: The lateral septum (LS) processes the affective significance of sensorial information from the hippocampus and directs its outputs towards hypothalamic areas important for motivated, goal-directed behavior. The LS is one of the main afferents to the tuberomammillary nucleus of the hypothalamus (TMN), the only source of brain histamine. Both structures are

known to be implicated in mood and motivation. Furthermore, brain histamine is important in arousal and alertness. Novelty has motivational effects and can direct and reinforce behavior. We propose that the LS change the activity of histaminergic neurons, thus modifying histamine release through GABAergic inputs. The reduction of GABAergic input to the TMN should therefore increase vigilance. Here, we demonstrated, using immunocytochemistry and electron microscopy that LS inputs to the TMN region are indeed GABAergic, and that these terminals make symmetric synaptic contacts with other GABAergic terminals that do not come from of LS. We measured extracellular histamine and GABA levels in the LS using microdialysis and simultaneously obtained EEG recording. Reverse microdialysis into the LS with 0.1mM picrotoxin and 10mM glutamate increased histamine and GABA release in LS as well as increased wakefulness measured with EEG and frequency theta wave.

Disclosures: P. Farias: None. **J. Diaz:** None. **A. ocampo:** None. **J. Valdes:** None. **F. Torrealba:** None.

Poster

722. Mechanisms of Attention II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 722.06/AA2

Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant BCS- 1156601

Title: Where and how are relevant sensory signals for perceptual decisions selected in the brain and mapped onto appropriate actions?

Authors: *J. DITTERICH;
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Abstract: In everyday life, decisions based on sensory stimuli are usually made in the presence of an abundance of irrelevant sensory information. Successful decision-making therefore requires effective selection of decision-relevant information. To address the question where and how the brain separates decision-relevant from irrelevant sensory information and then maps the relevant information onto appropriate actions, we trained monkeys to make perceptual judgments about the direction of motion in random-dot motion stimuli that contained both a decision-relevant and an irrelevant motion component. The two components were orthogonal to each other, one horizontal (left or right), one vertical (up or down). A cue at the beginning of each trial indicated the relevant axis of motion for the current decision. Two choice targets appeared

randomly along one of the two possible diagonals, and the animals were trained to make an eye movement to the target that was most closely aligned with the relevant direction of motion. While challenging, we successfully trained monkeys to perform this task. Based on behavioral data collected so far, the relevant sensory information can have up to five times more impact on the monkey's choice than the irrelevant information. We recorded spiking activity and local field potentials from cortical areas MT and LIP, when possible, simultaneously. MT has previously been shown to carry information about the sensory evidence in motion discrimination tasks, whereas LIP carries decision-related activity. Microstimulation in either area can systematically bias the animal's choices. Based on the data we have been able to collect so far, neurons in MT respond almost identically to the decision-relevant and irrelevant motion components, indicating that neural activity is not substantially modulated by decision relevance. In contrast, ramping responses in LIP during evidence accumulation clearly reflect modulation of the motion signals according to decision relevance. The major selection of decision-relevant sensory evidence therefore apparently happens after or while the motion representation in MT is read out, but before or during the evidence accumulation process that is reflected in LIP's spiking activity. I will address the question whether simultaneously recorded neural activity provides insight into the selection process, including the possibility of reflecting decision relevance-related changes in functional connectivity.

Disclosures: J. Ditterich: None.

Poster

722. Mechanisms of Attention II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 EY025103

NIH F30 MH102010

Title: Spatiotemporal characterization of perisaccadic receptive field structure and attentional modulation in area V4

Authors: *A. C. MARINO[1,2], J. A. MAZER[3,1,4];
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Abstract: Every eye movement changes the subset of neurons in visual cortex representing specific features in the visual scene. Despite frequent saccades and resulting disruptions of activity patterns in retinotopically organized cortical areas, visual perception is remarkably stable. Studies have suggested that perceptual stability is facilitated by pre-saccadic remapping of receptive fields (RFs) in several (mostly parietal) visual areas. Classical remapping translates the RF parallel to the saccade vector, towards the "future field". Other studies, particularly in V4 and FEF, showed remapping can shift RFs towards the saccade endpoint, a possible substrate for perisaccadic compression and explanation for enhanced visual processing at saccade endpoints. Behavioral and fMRI experiments in humans have shown spatial attention can be sustained at both spatiotopic (i.e., specific scene features independent of gaze angle) and retinotopic locations across saccades and suggest the neural representation of attention, like visual perception, may be remapped in preparation for or response to saccades. To investigate RF and attentional remapping in single neurons we trained macaques to perform a novel behavioral task requiring them to sustain attention at a cued, spatiotopic location and respond to targets at this attended location while ignoring distractors at nearby, unattended locations and making guided saccades. Throughout each trial, low contrast, behaviorally irrelevant dynamic probe stimuli were used to construct a detailed characterization of the spatiotemporal receptive field (SRF) as a function of attentional state and time relative to saccade onset. Data were recorded from 103 sites (60 single- and 43 multi-unit) in V4 of two animals. Behavioral analysis shows that monkeys are able to reliably sustain a spatiotopic locus of attention while making saccades. During the fixation period, before animals were instructed to execute the saccade, 47% of sites exhibited significant attentional modulation of responses to probe stimuli, defined as increased (70%) or decreased (30%) evoked responses to probes when attention was directed into the RF. In the majority of sites, responses to probes appearing in the 100ms prior to saccade onset reveal little or no evidence of classical remapping. In many cells, examination of saccade-locked responses yield a diverse pattern of saccadic suppression, the extent and time course of which are dependent on the saccade vector, suggesting that activity in V4 reflects a complex interaction between oculomotor planning, execution, and bottom-up visual inputs.

Disclosures: **A.C. Marino:** None. **J.A. Mazer:** None.

Poster

722. Mechanisms of Attention II

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Topic: F.02. Animal Cognition and Behavior

Support: Supported by DFG-CRC-889

Title: Is spatial attentional gain modulation in area MT of primate visual cortex mediated by the cholinergic system?

Authors: *V. K. VEITH[1], C. QUIGLEY[1], S. TREUE[1,2];
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Abstract: Attentional modulation of sensory responses in extrastriate visual cortex of primates is characterized by gain changes, which are multiplicative changes of firing rates for a given combination of stimuli in the receptive field and the attentional state of the animal. There is evidence that the cholinergic system may be responsible for such changes in local neuronal responsivity. Herrero et al. (2008) showed that acetylcholine mediates attentional modulation in striate cortex through muscarinic receptors. Here we examined whether cholinergic influences can account for attentional modulation in extrastriate visual area MT, where neurons exhibit strong tuning for the direction of motion in their receptive field and firing rates are reliably enhanced by allocation of spatial and feature-based attention. We recorded from single cells in two awake, behaving rhesus monkeys while they performed a spatial attention task. Stimuli were two random dot patterns that were presented on a computer screen, one inside the recorded cell's receptive field and the other in the opposite visual field. In each trial, the monkey was cued to attend to one of the stimuli and to report a direction change in the cued stimulus only. We compared firing rates when the moving stimulus in the receptive field was attended vs. unattended to quantify gain changes by spatial attention. During recordings, we used pressure injection to pharmacologically manipulate the direct vicinity of the recorded neuron in a block-wise fashion. We used the antagonist scopolamine to block the muscarinic or nicotinic cholinergic receptor subtype respectively, or the agonist acetylcholine to increase its extracellular concentration. The pattern of attentional modulation during injection was compared to baseline blocks recorded before injection. Control injections with saline solution did not affect firing rate, ruling out an effect of the injection process per se on firing rates. As expected, directing spatial attention into the receptive field of a recorded neuron significantly increased responses (by about 18%) in both monkeys. The muscarinic antagonist scopolamine led to an increase or decrease in firing rate and reduced this attentional modulation (to about 12%) in both monkeys. So far this trend has not reached significance. First results from injecting the direct agonist acetylcholine show increased firing rates and indicate an increase in attentional modulation (from about 18% to 21%). Therefore our data suggest but do not yet prove an involvement of muscarinic receptors in mediating response modulations in area MT by spatial attention.

Disclosures: V.K. Veith: None. **C. Quigley:** None. **S. Treue:** None.

Poster

722. Mechanisms of Attention II

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Topic: F.02. Animal Cognition and Behavior

Support: ANR grant 14-CE13-0005-01

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James S. McDonnell Foundation

Title: Modulating the noradrenergic system at rest

Authors: C. GUEDJ[1,2], E. MONFARDINI[1,3,2], A. REYNAUD[1,2], A. FARNÈ[1,2], M. MEUNIER[1,2], *F. HADJ-BOUZIANE[1,2];
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Abstract: The locus cœruleus (LC) is the principal source of noradrenaline (NA) in the brain and this neuromodulator is known to influence attentional processes. Yet, to date, the exact mechanisms by which NA acts upon the neural network sub-serving attention remain poorly understood. Current models postulate that LC activity acts as an ‘‘interrupt’’ signal (Dayan and Yu, 2006) or as a ‘‘network reset’’ signal (Bouret and Sara, 2005) that allows flexible and optimal reconfiguration of brain activity. Here, we used resting-state fMRI to investigate the functional connectivity pattern of the LC following a systemic delivery of an NA-reuptake inhibitor that enhances the level of NA in the brain. We scanned three monkeys in a 1.5 T scanner using a contrast agent (MION) while they were sitting motionless in the dark (400TRs, TR=2s, voxel size=2x2x3mm). We compared the changes induced by intramuscular injections of either saline or a NA-reuptake inhibitor (atomoxetine, 0.5 mg/kg) on the LC functional connectivity using a seed-based approach. First, in the saline condition, we found that the LC activity was strongly correlated with activity in the anterior cingulate cortex, the ventral and lateral thalamic nuclei, the somatosensory cortex (areas 1-2 and 3 a/b) and the cerebellum. This connectivity pattern fits well with the known anatomical projections of the LC neurons. Second, compared to the saline injection, the injection of atomoxetine selectively increased the strength of connectivity between the LC and the intraparietal sulcus (area LIP), the amygdala and regions within the superior temporal sulcus while it decreased the strength of connectivity between the LC and somatosensory cortex (areas 1-2). Our preliminary results suggest that the injection of atomoxetine affects the strength of connectivity between the LC and a very specific network of

brain regions, including LIP and the amygdala, two brain regions important in encoding the saliency of stimuli in our environment. It is thus possible that enhancing NA availability alters LC activity (Bari and Aston-Jones, 2013) that might in turn orchestrates brain state transitions through LIP and the amygdala, to optimally adjust attentional resources to salient incoming stimuli.

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Poster

722. Mechanisms of Attention II

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Support: Santos Dumont Institute

AASDAP

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INCEMAQ (Programa INCTs do CNPq/MCT)

FAPERN

CAPES

CNPq

Title: An open-source and low-cost operant conditioning equipment for studying auditory discrimination in common marmosets

Authors: *M. W. RIBEIRO, J. F. R. NETO, F. L. BRASIL, M. F. P. ARAÚJO;
Edmond and Lily Safra Intl. Inst. of N, Macaiba, Brazil

Abstract: Common marmosets (Callithrix jacchus) are small New World primates that share several social, cognitive and behavioral characteristics with humans, such as cooperative breeding and a rich vocal repertoire. Therefore, understanding how the marmoset brain processes sounds, particularly vocal stimulus, can shed light into the mechanisms involved in language processing and evolution. One approach for studying vocal processing in marmosets can be

operant conditioning tasks requiring the discrimination of sound stimuli. Such tasks allow the control and manipulation of experimental variables in order to relate a specific behavior to a neural activity. The aim of this study was to develop an open-source hardware and software operant conditioning box for marmosets designed to output sound stimuli, and input motor responses. The setup consists of a plexiglass box (25x25x45cm) placed inside a double wall acoustic chamber. The back side of the box has two apertures through which retractable metal bars can be presented to the animal. Each bar is connected to a linear servo (Futaba S-148 with an EMS Linear servo Conversion Kit For Futaba S-148 and S-3151 Servos). The bars were also connected to a LN555 circuit (Texas Ins) that detects a touch and sends output signal to the controller. A recipient for reward delivery was positioned between the bars. The reward system was driven by a DC motor controlled by an H bridge LN298. The box controller was an Arduino Mega (Arduino, Italy) with a sound player shield (MP3 Player Shield, Spark Fun Inc) stacked above a custom made open source shield that controls the sound stimuli and receives the response from the bars to release the reward in correct trials. The sound shield played sound stimuli (7 kHz, 5 sec, 70 dB level) through a high fidelity speaker (D220Ti, JBL) powered by an amplifier (Versátil Mono 100W, Hayonik). The open source custom operant conditioning box showed high temporal precision to control input and output for auditory discrimination tasks with marmosets.

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Poster

722. Mechanisms of Attention II

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Topic: F.02. Animal Cognition and Behavior

Support: ＪＳＰＳ Research Fellowships for Young Scientists

Title: Attention-accumulation process in gaze behavior during multi-cue decision making with manual response and free eye-movement

Authors: *R. AKAISHI, E. HOSHI;
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Abstract: The eye-movement is known to reflect the internal process going on in the brain. The gaze behavior has been widely used as an instrument to obtain reward in research of decision

making. However, in natural environment of primates, the manual movement is the instrument to obtain the reward and the eye is the device to gather the information to predict the rewarding event as a result of action. To re-examine the significance of eye movement in more ecologically valid situation, we created a novel decision making task with free eye-movement and manual response to indicate the choice of action target based on multiple instructing cues. Here, we show that the eyes were increasingly more likely to be fixated on the chosen action target as the timing of response made by the hand approached though hand action was an instrument for obtaining reward in the task. The frequency of the fixation on the chosen target roughly converged on the common peak value of fixation frequency. This accumulating pattern of gaze to the chosen target coexisted with the competing pattern of fixation on the unchosen target and the divergences of the two accumulating processes were dependent on the information given by the cues and correlated with the timing of target touch. Furthermore, eyes tended to be attracted to the cues that instructed the action consistent with the actually chosen target. But these fixations on the cues were usually brief and preceded or overlapped with the earlier part of the accumulating pattern of the fixation on the action targets. These results of gaze behaviors suggest that the rapid attentional process select the information relevant for action selection and the selected information is fed into the more deliberate accumulation process that chooses the action leading to a reward. The results may also have implications for the interpretations of neural signals recorded during the decision making tasks by favoring the view of accumulating saliency signal.

Disclosures: **R. Akaishi:** None. **E. Hoshi:** None.

Poster

722. Mechanisms of Attention II

Location: Hall A

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Program#/Poster#: 722.12/AA8

Topic: F.02. Animal Cognition and Behavior

Title: Response properties of neurons in the pigeon NCL during a perceptual decision making task

Authors: ***R. PUSCH**[1], J. PACKHEISER[1], O. GÜNTÜRKÜN[1], M. C. STÜTTGEN[2];
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Abstract: Executive functions consider external as well as internal conditions to govern cognitive processes and elicit adaptive behavior. The process of decision-making requires an integration of multimodal sensory information in order to successfully evoke appropriate

choices. In mammals, the prefrontal cortex (PFC) is the key structure involved in the execution of these cognitive processes. Recent studies conducted with avian species revealed that birds are on par with mammals regarding these cognitive processes. Despite differences in its anatomical structure the Nidopallium caudolaterale (NCL) has been hypothesized as the functional analogue to the PFC enabling birds to perform tasks that require higher-level cognition as decision making, even though it has developed through a process of convergent evolution independently from mammals. To gain insights into general neuronal principles which might be comparable across different vertebrate classes, we recorded single cell activity in the NCL during perceptual decision making. Pigeons (Columba livia) performed a single interval forced-choice categorization task. We manipulated the difficulty of the task by reducing the difference in luminance for some stimuli and further introduced different reward contingencies for a stimulus subset. While performing the tasks, single unit activity was recorded. The majority of the recorded units were responsive to different phases of the experimental task: During the sample phase a fraction of neurons exhibited differential responses in regard to the stimulus shown. For some units these responses were signaling an upcoming choice of the animals in a subsequent phase of the experimental task. A subpopulation discriminated in addition between stimulus identities. Similar results were obtained for a following delay phase. In this phase of the experimental paradigm no sensory cues were available for three seconds. Neuronal responses of some units maintained a distinctive response pattern indicating a forthcoming decision in the choice phase. We further investigated our data with regard to a possible coding of reward-expectancy in the NCL. We discuss our findings in terms of the hypothesis that cognitive processes such as working memory or judgment of reward probability might be processed in a comparable way within birds and mammals supporting the assumption of functional homogeneity between the avian NCL and the mammalian PFC.

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Poster

722. Mechanisms of Attention II

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Topic: F.02. Animal Cognition and Behavior

Support: NIDCR DE022746

Title: Functional connectivity properties of the rat brain during conscious wakefulness and isoflourane-induced unconsciousness

Authors: *M. E. GHANTOUS, P.-C. CHANG, A. BARIA, V. APKARIAN;
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Abstract: Identifying properties of conscious brain activity constitutes an important challenge in neuroscience. Previous studies in monkeys have shown that different states of consciousness elicit distinct spatiotemporal functional connectivity patterns. Similarly, studies in humans have demonstrated that thalamo-cortical connectivity is a key mediator of conscious wakefulness. Here we investigate functional connectivity in the brains of rats during both conscious and anesthesia-induced unconscious states to determine how conscious wakefulness is reflected in synchronized activities across the whole brain. Resting state brain activity was recorded in rats (N=10) using functional magnetic resonance imaging (fMRI) for both vigilance states. Functional images were preprocessed and co-registered to a standard anatomical template of the rat brain and two connectivity analyses were performed. To examine whole-brain connectivity, rat brains were parceled into 96 regions of interest (ROI) based on a standard rat atlas. The blood-oxygen level dependent (BOLD) time series of each ROI was extracted, and a Z-Fisher transformed Pearson correlation coefficient for all pairs of ROIs was computed to generate correlation matrices for individual brains under both conditions of vigilance. Connectivity between all brain ROIs were compared across conditions using network-based statistics. Thalamic connectivity was specifically examined using a seed-based approach. Brains of awake rats showed an overall greater average connectivity across regions, with both stronger negative and positive correlations. Additionally, the awake brain exhibited more long-distance connections between anterior and posterior regions whereas sparser and more local connections were present under anesthesia. Moreover, the anesthetized brain showed reduced thalamic functional connectivity to the rest of the brain, a finding that corroborates the notion that thalamo-cortical connectivity is a mediator of consciousness. Overall, these findings suggest that different states of vigilance uniquely contribute to resting state functional connectivity. The results also emphasize the need of awake-based fMRI scanning in animals for translational research involving conscious-brain activity in humans. Funded by NIDCR DE022746

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Poster

722. Mechanisms of Attention II

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Topic: F.02. Animal Cognition and Behavior

Title: Space influences motor and visual cortical excitability: Effects of hand location on motor evoked potentials and eye position on phosphene thresholds

Authors: *H. B. COSLETT[1], M. DE WIT[2], O. FASEYITAN[3];
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Abstract: We performed two experiments to test hypotheses regarding effects of gaze and hand position on motor cortex excitability measured by TMS. First, we reasoned that if the left hemisphere is dominant for motor attention, one would expect that motor evoked potentials (MEPs) would be larger when the right or left hand is placed in the right side of space. Second, although the locations of gaze and action may be dissociated, they are usually linked; on the assumption that visual attention typically follows gaze, we predicted that gazing at the hand would increase MEPs as compared to when hand location and gaze were dissociated. Experiment 1 tested these predictions by measuring MEPs for the right hand of 20 right-handers in 4 conditions, generated by crossing the variables of side of hand (R/L) and direction of gaze (R/L) relative to the body midline. There was a main effect of side of gaze with MEPs larger on the right than left (107% vs 93%, $p < .01$). There was also an effect of congruence such that MEPs were larger when subjects did NOT gaze at their hand (105% vs 95%, $p < .01$). In Experiment 2 the effect of gaze was replicated with the LEFT hand of right-handers in that MEPs were larger for the left hand when gazing to the right side of space ($p<.03$). These data support the hypothesis that the left hemisphere is dominant for motor attention and that this is independent of effector. The data are INCONSISTENT with the hypothesis that effects of gaze-hand congruence and motor attention would be additive. One interpretation of the latter finding is that increased MEPs when gaze and hand position are dissociated reflects an increased need for surveillance of the environment outside of the field of gaze. If this is true, one would expect to observe analogous effects in other domains such as vision. In Experiment 3 we tested this prediction by measuring phosphene detection as a function of TMS intensity in the left visual field in subjects with their eyes closed but gazing ahead or to the right or left. Subjects were significantly MORE likely to detect phosphenes in the left visual field when directing their eyes to the right or center as compared to the left. That is, as in Experiments 1 and 2, cortical excitability as assessed by TMS was greater in brain regions corresponding to locations to which subjects did not direct their gaze. These data support two conclusions: first, the left hemisphere is dominant for action independent of effector; second, potentially distinct attentional systems may influence processing at attended locations as compared to non-attended locations; the latter may be manifested in part as changes in sensory-motor cortical excitability.

Disclosures: **H.B. Coslett:** None. **M. de Wit:** None. **O. Faseyitan:** None.

Poster

722. Mechanisms of Attention II

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH MH051383

Title: Neural correlates of subjective value in the nematode worm Caenorhabditis elegans

Authors: *A. W. KATZEN[1], W. T. HARBAUGH[2], S. R. LOCKERY[3];
[2]Econ., [3]Biol., [1]Univ. of Oregon, Eugene, OR

Abstract: The interaction of cost and perceived quality is central to value-based decision making. Such decisions are made by correctly assessing the costs and benefits of two or more courses of action and executing the one offering the highest value--meaning the greatest benefit--relative to the costs incurred. Given the constraint of a finite budget--whether that be time, effort, or money--calculating this tradeoff between cost and benefit is critical to any decision between competing options. Here, we show that the nematode worm Caenorhabditis elegans engages in such a cost-benefit analysis when choosing between food options in a decision making task. Worms shift their consumption between foods in response to changes in relative cost, and the subjective value of each food is dependent on the worm's prior experience with that food. Furthermore, we show that response properties of primary sensory neurons reflect the subjective value of available options. Future research could identify the locus of decision making and, given the experimental advantages of *C. elegans*, these studies could be among the first to move beyond observing neural correlates of decisions to the point of identifying their physiological and genetic causes.

Disclosures: A.W. Katzen: None. **W.T. Harbaugh:** None. **S.R. Lockery:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

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Title: Changes in neuronal activity in the nucleus accumbens core during the acquisition of instrumental behavior in rats

Authors: *M. VEGA VILLAR[1], K. L. CAREF[2], J. J. KIM[3], J. C. HORVITZ[5], S. M. NICOLA[4]; [1]The Grad. Center, CUNY, New York, NY; [2]Dominick P. Purpura Dept. of Neurosci., [3]Dept. of Psychiatry and Behavioral Sci., [4]Dominick P. Purpura Dept. of Neurosci. and Dept. of Psychiatry and Behavioral Sci., Albert Einstein Col. of Med., New York, NY; [5]Dept. of Psychology, City Col. of New York, CUNY, New York, NY

Abstract: A considerable body of research indicates that the nucleus accumbens (NAc) is critically involved in the acquisition and expression of reward-oriented behavior. This evidence points at the possibility that neuronal activity in the NAc might undergo changes as these behaviors are learned. However, most studies using electrophysiological recordings of the NAc focus on firing patterns associated with the expression of an already acquired behavior. The evolution of firing patterns in the NAc associated with the emergence of new behaviors has hardly been studied. Using *in vivo* electrophysiological recordings from the first to the last day of training, this study examined changes in neuronal activity in the NAc core as rats acquired a simple instrumental behavior (i.e. nose-poking into a receptacle when a cue indicated availability of sucrose solution). Using this technique, we identified neural correlates of different task-related events that developed as the conditioned approach response was acquired. The nature of these changing patterns, their relationship with different aspects of the incipient behavior and their significance are discussed.

Disclosures: M. Vega Villar: None. **K.L. Caref:** None. **J.J. Kim:** None. **J.C. Horvitz:** None. **S.M. Nicola:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

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Support: NIH Grant R00 DA024719

NIH Grant F32 DK102294

Title: Enhancement of operant responding for food reward by DREADD activation of pyramidal neurons projecting from the mPFC to the nucleus accumbens

Authors: *D. M. WARTHEN[1], K. L. GASSMANN[1], S. M. KHALIL[1], N. P. ROGERS[1], L. S. ZWEIFEL[2], M. M. SCOTT[1];
[1]Pharmacol., Univ. of Virginia, Charlottesville, VA; [2]Psychiatry and Pharmacol., Univ. of Washington, Seattle, WA

Abstract: The medial prefrontal cortex (mPFC) is implicated in a wide range of cognitive functions, including reward evaluation, decision making, memory extinction, mood, and task switching. The mPFC exerts its executive control function via projections composed exclusively of excitatory pyramidal neurons that innervate target areas throughout the brain. It is likely that specific projections mediate defined aspects of mPFC behavioral control. Projections from the mPFC to the nucleus accumbens (NAc), in particular, are likely important for top-down control of motivated behaviors. Thus, we sought to determine the behavioral effects of enhancing the excitatory outflow of projections exclusively from the mPFC to the NAc of mice. To accomplish this, we used a combinatorial viral approach, targeting the NAc with a canine adenovirus (CAV) expressing Cre recombinase, while simultaneously targeting the mPFC with an adeno-associated virus (AAV) encoding a Cre-dependent Gq-coupled Designer Receptor Exclusively Activated by Designer Drugs (DREADD). We found that increasing excitatory output from the mPFC to the NAc via DREADD selectively enhanced performance in a food rewarded operant assay, while having no impact on performance in non-contingent assays. Specifically, activation of NAc-projecting mPFC pyramidal neurons had no effect on binge-like consumption of a high fat diet, social interaction, social habituation and discrimination, behavior in an open field or elevated plus maze, or exploration of a novel object. Interestingly, operant performance was enhanced only when the mice were kept on food restriction and not when they were satiated. As our prior work showed that non-selective stimulation of mPFC pyramidal cells enhanced operant responding in the fed state, it is likely that PFC projections to areas other than the NAc are also necessary to enhance responding in the sated animal.

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Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.03/AA14

Topic: F.02. Animal Cognition and Behavior

Support: R01 DA025983

Title: Role of the Core of the Accumbens in Operant Acquisition

Authors: *S. JONKMAN, P. KENNY;
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Abstract: The brain regions that mediate the acquisition of operant responding have not been clearly defined to date. It has been proposed that the core region of the Nucleus Accumbens plays an important role, but the available experimental evidence is unequivocal. Local post-learning infusions of anisomycin have been shown to reduce lever pressing for food. However, subsequent work showed that intra-Accumbens anisomycin after food consumption produced a conditioned taste avoidance, presumably due to aversive effects of intra-cranial anisomycin. When these aversive side-effects were controlled for, operant acquisition was not affected by anisomycin infusions. Here we revisit this question by having rats acquire a lever press response for food in one single session, and testing for operant learning two days later in extinction conditions. We ensure that anisomycin did not affect operant reinforcer valuation by interposition of a novel food reward between operant learning and anisomycin infusions, effectively overshadowing any association between the operant reward and negative effects of intra-cranial anisomycin. We confirm that anisomycin significantly reduced subsequent consumption of the novel reward, while leaving the valuation of the operant rewards unchanged. In terms of the overall number of lever presses in the test session, anisomycin infusions did not reduce lever pressing compared to either saline or 6 hrs delayed anisomycin infusions. Based on pilot experiments, we then analyzed the lever press data in terms of the clustering of responses. Control animals engaged both in isolated lever presses and in more sustained series of lever presses that we define as bouts. Anisomycin infusions in the core of the Accumbens significantly reduced both the number of lever presses in a bout, and the percentage of lever presses that turned into a bout versus a solitary press. These comparisons were significant for both the saline and delayed anisomycin control groups. Thus, these results confirm that plasticity in the core of the Nucleus Accumbens does not play an important role in operant learning per se, but rather affects the emergence of sustained operant responding. These results are broadly consistent with the finding that dopamine activity in the core of the Accumbens is necessary for sustained responding in high ratio and progressive ratio testing. We extend those results to suggest that local plasticity is important for learned motivation.

Disclosures: S. Jonkman: None. **P. Kenny:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: Wenzhou Medical University (No.89211010; No.89212012)

the Zhejiang Provincial Special Funds (No.604161241)

NIH grants (NS041083-11, NS073947)

Title: Opotogenetic activation of adenosine A2A receptor signaling in the dorsomedial striatopallidal neurons suppress goal-directed behavior

Authors: *J.-F. CHEN[1,2], Y. LI[2], Y. HE[2], M. CHEN[2], L. CHEN[3], P. LI[4], B. LI[2], H. LI[2], Z. HUANG[3], Z. LI[2];
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Abstract: Striatum plays an essential role in neural control of goal-directed and habitual behavior to achieve optimal performance of task. Adenosine A2A receptors (A2ARs) with enriched expression in the striatum modulate instrumental learning, but the contributions of the striatopallidal A2ARs in dorsolateral (DLS) and dorsomedial striatum (DMS) to the control of instrumental learning are not defined. To address whether the transient activation of the striatopallidal A2AR in DMS and DLS precisely at the time of reward was sufficient to modulate instrumental learning, we have developed the rhodopsin-A2AR chimeras (opto-A2AR) and demonstrated that transient light activation of A2AR signaling in the striatopallidal neurons in a "time-locked" manner precisely at the time of reward did not affect instrumental learning at the acquisition or extinction phases, but was sufficient to change animal's sensitivity to outcome devaluation. Specifically, optogenetic activation of striatoapllidal A2AR signaling in DMS suppressed goal-directed behaviors while focally genetic knockdown of striatopallidal A2ARs in DMS enhanced goal-directed behavior by the devaluation test. By contrast, optogenetic activation or focal AAV-Cre-mediated knockdown of striatopallidal A2AR in DLS had little effect on instrumental learning. Thus, the striatopallidal A2AR signaling in DMS exerts inhibitory control of goal-directed behavior by acting precisely at the time of reward, and may represent a therapeutic target to reverse abnormal habit formation that is associated with compulsive obsessive disorder and drug addiction.

Disclosures: **J. Chen:** None. **Y. Li:** None. **Y. He:** None. **M. Chen:** None. **L. Chen:** None. **P. Li:** None. **B. Li:** None. **H. Li:** None. **Z. Huang:** None. **Z. Li:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: Australian Research Council Laureate Fellowship (Prof Bernard Balleine)

Australian Research Council Discovery Project Grant

National Health and Medical Research Council Project Grant

Title: The goal-directed pathway: A bilateral corticostriatal pathway mediates the acquisition of goal-directed actions

Authors: ***G. HART**, L. A. BRADFIELD, S. Y. FOK, B. W. BALLEINE;
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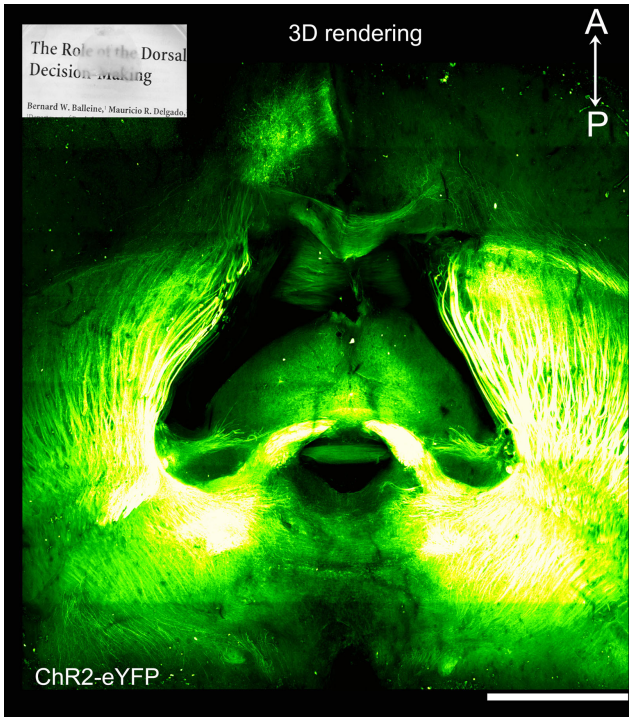
Abstract: In the rat, the prelimbic (PL) region of the medial prefrontal cortex and the posterior dorsomedial striatum (pDMS) are critical for the acquisition of goal-directed actions. The PL has dense glutamatergic projections to the DMS arising from two subpopulations of pyramidal projection neurons; intratelencephalic (IT) neurons, that project ipsilaterally, contralaterally and bilaterally to the striatum, and pyramidal tract (PT) neurons that project to the brainstem via the pyramidal tract, with ipsilateral collaterals in the striatum. Our aim was to investigate the role of ipsilateral and bilateral PL to pDMS projections in the acquisition of goal-directed behavior. We used a recently published tissue clearing procedure (CLARITY; Chung et al., 2013) to map the bilateral projection pathway of PL neurons to the DMS in an intact rat brain (Figure 1, scale bar 2mm, ventral aspect). We then used instrumental training and outcome devaluation tests to assess the functional role of ipsilateral and contralateral PL to pDMS projections in goal-directed behavior. We infused a trans cellular tracer protein; wheat germ agglutinin (WGA) fused to a virus expressing the Cre recombinase (Gradinaru et al., 2010) into the pDMS unilaterally, and performed a cell-body lesion of the PL in the ipsilateral hemisphere. Retrogradely infected IT neurons in the contralateral PL were then selectively infected with a Cre-dependent Gi-coupled M4 DREADD virus (Armbruster et al., 2007). We used Clozapine-N-Oxide to temporarily silence contralateral and bilateral projecting IT neurons during instrumental training. We found

that goal-directed instrumental actions are acquired in a bilateral (IT) PL to pDMS pathway; rats lacking bilateral IT neurons during instrumental training failed to show goal-directed performance on test. Results are discussed in terms of the role of this pathway in top-down modulation of lateralized habit circuits to control striatal output in each hemisphere and produce cohesive action.



Disclosures: **G. Hart:** None. **L.A. Bradfield:** None. **S.Y. Fok:** None. **B.W. Balleine:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

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Topic: F.02. Animal Cognition and Behavior

Support: DGAPA-PAPIIT IN209911

CONACyT 152208

Technical assistants Angela Gabriela Vera

Technical assistants Alejandro Rangel-Hernández.

English revision Shaun Harris

Title: The effect of appetitive context pre-exposure on inhibitory avoidance learning

Authors: *M. J. OLVERA-CALTZONTZIN, M. MIRANDA;
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Abstract: Memory consolidation has been studied by different learning models, particularly using aversive tasks as inhibitory avoidance (IA), where context is associated with an electric shock, and conditioned taste aversion (CTA), where taste is associated with gastric malaise. Context and flavor are directly interacting during learning, thus the aim of this research was to study how these two types of stimuli are learning together, and if they potentiated or inhibited IA learning. Accordingly, we evaluated if the consumption of appetitive stimulus during IA training has an effect on the aversive learning; we also assessed the effect of context pre-exposure with this hedonically reinforcer (i.e. sugar) on IA latent inhibition. For this purpose, some rats were pre-exposed to a new context (IA chamber), and allowed to drink a sugar solution (10% in water) inside the dark compartment of the chamber. Next day, IA training was conducted with or without sugar presentation and 24 h later IA memory was evaluated. The results demonstrated that IA chamber pre-exposure, whether reinforced or not, produced a significant reduction in the entrance latency to the dark compartment the next day. During IA memory retrieval session, animals trained in a reinforced context showed a significant decrement in the latency to enter the dark compartment of the chamber during IA test retrieval, however, animals pre-exposed or directly trained in IA, without the hedonic reinforcer, had higher latencies during memory retrieval, indicating a stronger IA. These results give evidence about the interactions between flavor and context, and indicate that context learning is modulated by the hedonic value of the experience.

Disclosures: M.J. Olvera-Caltzontzin: None. **M. Miranda:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

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Program#/Poster#: 723.07/AA18

Topic: F.02. Animal Cognition and Behavior

Support: PSI2013-44945-P Ministerio de Economía y Competitividad

HUM-642 Junta de Andalucía

Title: Symmetrical transfer effects between instrumental and consummatory tasks in rats selected for Low Avoidance/High Anxiety

Authors: *C. TORRES[1], L. CUENYA[2], M. SABARIEGO[3], R. DONAIRE[1], A. FERNÁNDEZ-TERUEL[4], M. R. PAPINI[5];
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Abstract: Transfer effects refer to the influence of training in one situation on performance in a subsequent situation. Transfer effects have been described in a variety of situations, including tasks involving reward loss. In this experiment, inbred Roman strains extensively selected for high vs. low avoidance learning were exposed to two tasks in a counterbalanced order. Extensive research on these strains demonstrates that low-avoidance rats exhibit higher levels of anxiety in a wide range of situation than high-avoidance rats. Moreover, previous research showed that repeated reward loss increases resilience in low-avoidance/high-anxiety rats, but not in high-avoidance/low-anxiety rats. Therefore, we predicted that only low-avoidance/high-anxiety rats exposed to reward devaluation in one situation would show transfer of resilience to a different reward devaluation task. One task was instrumental successive negative contrast (iSNC) and the other consummatory successive negative contrast (cSNC). In the iSNC task, rats were reinforced with either 12 or 2 pellets in the goal box of a runway for five 6-trial sessions and then all animals received 2 pellets for an additional six 6-trial sessions (a 12-to-2 pellet downshift). Response latency (in seconds) was the dependent measure. In the cSNC task, rats received access to either 22% or 4% sucrose for sixteen 5-min sessions and then all animals received access to 4% sucrose for an additional four sessions (a 22-to-4% sucrose downshift). Sucrose intake (in milliliters) was the dependent variable. Unshifted control groups were included in both tasks. These tasks differ in the involvement of the hippocampus, which plays a critical role in iSNC, but apparently not in cSNC. As predicted, low-avoidance/high-anxiety rats showed both iSNC and cSNC effects when first exposed to these task (i.e., without previous reward-loss experience), whereas both phenomena were absent in transfer animals previously exposed to the alternative task. Control groups were not affected by previous experience. By contrast, high-avoidance/low-anxiety rats did not exhibit the iSNC effect either when first exposed or after exposure to the cSNC task. This prevented the study of transfer effects in this strain. Despite extensive psychogenetic selection for low-avoidance/high-anxiety behavior (since the 1960s), experience with reward devaluation in one situation immunizes these animals against the frustration induced by reward devaluation in a different situation. These results open an opportunity to study the neurobiology of resilience in animals prone to emotional vulnerability.

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Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.08/AA19

Topic: F.02. Animal Cognition and Behavior

Support: Shire Development, USA

Title: Binge eating rats exhibit compulsive and perseverative behaviour in a novel food reward/punished responding model

Authors: ***D. J. HEAL**[1,2], P. HUTSON[2], S. GODDARD[1], R. BRAMMER[1], S. VICKERS[1]; [1]RenaSci Ltd, Nottingham, United Kingdom; [2]Shire Develop. Inc, Wayne, PA

Abstract: Binge-eating disorder (BED) is a psychiatric condition characterised by repeated episodes of compulsive, excessive consumption of palatable foods. A rat model of binge-eating (BE) has been validated in which rats are given irregular, limited access to chocolate (Vickers et al, 2013, SfN abst 236.03). We have now developed a novel food reward/punished responding model to determine whether BE rats show compulsive and perseverative behaviours when given access to chocolate. Adult, female, Wistar rats (n=34) with continuous access to normal chow and water were given intermittent, 2 hr access to chocolate over 28 days. Non-binge (NB) controls were given an empty pot on the binge days. BE and NB rats were trained in the conditioned avoidance response (CAR) test in a 2-chamber shuttle box, ie a tone/light stimulus warned of a mild foot shock 10s later if the rats did not move to the adjacent compartment. After rats were proficient in the CAR test, the model was altered to a food reward/punishment conflict test. A jar was placed in 1 compartment of the shuttle-box (chocolate for BE rats; empty for NB rats). If the rat entered the chamber with the pot, the conditioning stimulus was presented after a variable interval and if the rat did not leave it received a foot shock10s later. Occupancy of the "safe" chamber with no pot in it did not initiate a trial or administration of foot shocks. BE rats developed robust chocolate bingeing with concomitant reductions in chow intake and maintained normal body weight. BE rats consumed ~45% of their daily food intake in the 2 hr binges. There was no difference between the BE rats and NB controls for the rate of acquisition or proficiency in performing the CAR test. In the reward/punishment conflict test, BE rats consumed 35.2 ± 5.2 kJ of chocolate. BE rats spent 309 ± 14 sec in the chocolate paired compartment (~74% of the session) compared with 237 ± 10 sec (~20%) for NB controls ($p<0.05$). BE rats responded to the

conditioned stimulus and left before receiving a foot shock (avoidance) in 78% of the trials compared with 98% for NBs ($p<0.01$). The percentage of trials when BE rats received foot-shocks before leaving the compartment (escape) was 10 fold higher than NBs ($p<0.01$). The mean total escape time when BE rats tolerated foot shocks and remained in the chocolate compartment was ~60 fold greater than the NB controls ($p<0.01$). BE rats showed compulsive and perseverative behaviours in the food reward/punishment conflict test. BE rats were unable to resist entering the chocolate-paired compartment even though the consequence of initiating a trial was potentially to receive a foot-shock. BE rats were also prepared to receive more foot-shocks than NB controls.

Disclosures: **D.J. Heal:** 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.; Shire Development, PA, USA. **P. Hutson:** None. **S. Goddard:** None. **R. Brammer:** None. **S. Vickers:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

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Program#/Poster#: 723.09/AA20

Topic: F.02. Animal Cognition and Behavior

Support: Shire Development, USA

Title: Binge-eating rats show marked impulsivity in a delay discounting test

Authors: *S. P. VICKERS[1,2], P. HUTSON[2], S. GODDARD[1], M. HALLAM[1], R. BRAMMER[1], D. HEAL[1];
[1]Renasci Ltd, Nottingham, United Kingdom; [2]Shire Develop. Inc, Wayne, PA

Abstract: Binge-eating disorder (BED) is a psychiatric condition characterised by repeated, excessive consumption of palatable food. Impulsivity and a loss of inhibitory control are important factors in BED (Schag et al, 2013, PLoS One 8: e765421). A rat model of binge-eating (BE) has been validated in which rats are given irregular, limited access to chocolate (Vickers et al, 2013, SfN abst 236.03). We have tested BE rats in a variant of the delay discounting task to determine whether they exhibit impulsive behaviour when given access to chocolate. Adult, female, Wistar rats (n=42) with continuous access to chow and water were trained to lever press for chocolate pellets in a delay discounting task. One lever delivered a single chocolate pellet

immediately while the other delivered a 3 pellet reward with increasing delay after every 5th trial, ie 0, 4, 8, 16, 32 sec. Rats were divided in 2 groups, ie BE rats given intermittent 2 hr access to chocolate over 28 days and non-binge (NB) controls given an empty pot on binge days. Both groups were tested in delay discounting on Days 17-18 and Days 30-31. BE and NB rats gained weight at the same rate. BE rats developed robust chocolate bingeing after ~2 weeks with concomitant reductions in chow intake. BE rats consumed ~50% of their daily food intake in the 2 hr binges. The NB controls showed a decreasing preference for the larger 3 pellet reward as the delay was increased on Days 17-18 [% responses on 3 pellet lever: 0 sec = 70.1±8.3; 4 sec = 57.6±5.4; 8 sec = 51.3±8.2; 16 sec = 38.9±6.7; 32 sec = 17.8±4.9; overall = 46.1±3.4] and Days 30-31 [0 sec = 71.8±11.0; 4 sec = 48.8±5.1; 8 sec = 33.0±6.9; 16 sec = 42.3±9.2; 32 sec = 19.9±5.8; overall = 42.4±5.6]. The responses of the NB rats did not vary over the course of the study. In contrast, BE rats showed significantly lower preference for the delayed larger reward as binge-eating became more established and their level of impulsivity increased. Compared with NBs, BE rats showed enhanced delay discounting on Days 17-18 [% responses on 3 pellet lever: 16 sec = 23.5±5.7, $p<0.05$; overall = 37.3±4.1, $p<0.05$] and at several delay intervals on Days 30-31 [% responses on 3 pellet lever: 8 sec = 17.1±4.5, $p<0.05$; 16 sec = 16.7±4.1, $p<0.01$; overall = 23.3±3.9, $p<0.001$]. BE rats consumed the same number of pellets as NB rats in the tests on Days 17-18, but 10% fewer ($p<0.05$) on Days 30-31. BE rats showed an unequivocal lack of tolerance to delay for larger chocolate rewards in the delay-discounting task when compared against NB controls. This intolerance of delayed reward, characteristic of impulsive behaviour, resulted in BE rats preferring to press for the smaller immediate reward even if it resulted in them consuming less chocolate.

Disclosures: **S.P. Vickers:** 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.; Shire Development, PA, USA. **P. Hutson:** None. **S. Goddard:** None. **M. Hallam:** None. **R. Brammer:** None. **D. Heal:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.10/AA21

Topic: F.02. Animal Cognition and Behavior

Support: Kansas State University USRG grant

Start-up funds-Kansas State University

Title: Individual differences in voluntary alcohol consumption predict operant extinction, but not devaluation, in rats

Authors: *H. FISHER, A. PAJSER, C. L. PICKENS;
Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: Alcohol use is associated with impaired decision-making. However, it is unclear whether alcohol use can lead to neurological changes that impair decision-making, or whether impaired decision-making might lead to the decision to consume alcohol. To investigate this question, we gave male Long-Evans rats chronic intermittent access (CIA) to alcohol (24-h access to 20% alcohol 3X per week) or water alone for 6 weeks during adolescence and early adulthood (PND 26-66) to examine the effects of alcohol consumption. Ten days after the final alcohol access day, rats were trained and tested in an operant devaluation task. We gave operant training using two levers, available during 40-second trials, with lever presses on the two levers earning different food reinforcers on an intermittent reinforcement schedule. After the rats received four days of training, two days for each lever, one of the reinforcers was devalued through selective satiety and devaluation was assessed through a choice test in extinction. The rats exhibited a large devaluation effect during the first five trials, with an abrupt extinction-induced reduction in responding on the nondevalued lever on the sixth trial. There were no differences in devaluation performance between the alcohol and water rats, and no correlation between devaluation performance and average alcohol intake during the last 2 weeks of alcohol access. However, alcohol intake was correlated with extinction responding, such that higher average alcohol intake was associated with a larger extinction-related decrease in responding on the nondevalued lever. These results suggest that individual differences in response inhibition, as seen in extinction, co-occur with different levels of motivation to consume alcohol. Additionally, our results provided no evidence that alcohol access altered devaluation or extinction. Our future studies will examine possible brain substrates related to this correlation between motivation to consume alcohol and operant extinction learning.

Disclosures: H. Fisher: None. **A. Pajser:** None. **C.L. Pickens:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.11/AA22

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 1DP2DA035149-01

Title: Cholinergic interneurons bidirectionally control extinction learning for a cocaine associated context

Authors: *J. C. FINKELSTEIN[1], J. LEE[2], I. WITTEN[2];
[2]Princeton Neurosci. Inst., [1]Princeton Univ., Princeton, NJ

Abstract: Context learning is an integral, but poorly understood, component of addiction. We have previously demonstrated a role for cholinergic interneurons in the nucleus accumbens in the acquisition of cocaine context associations. Here, we show that modulation of the firing activity of these neurons drives bidirectional control of extinction of a drug associated context. Using a cocaine conditioned place preference task, we found that optogenetically inhibiting cholinergic interneurons with eNpHR3.0-YFP during the first extinction test after conditioning increased the preference for the drug associated chamber relative to the YFP-only control group (repeated measures ANOVA, main effect opsin, $F(1,18)=6.372$, $p<.05$, n=10 NpHR3.0-YFP, n=9 YFP-only). In contrast, exciting these neurons with ChR2 diminished preference for the drug associated chamber (repeated measures ANOVA, main effect opsin, $F(1,17)=5.141$, $*p<.05$ n=10 ChR2-YFP, n=8 YFP). These changes in preference persisted throughout subsequent extinction trials without further manipulation of the cholinergic interneurons, suggesting that these neurons are both necessary and sufficient for extinction learning of a cocaine associated context.

Disclosures: J.C. Finkelstein: None. **J. Lee:** None. **I. Witten:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

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Program#/Poster#: 723.12/AA23

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 1DP2DA035149-01

Title: Comparing dopamine signaling in dorsal and ventral striatum during a reinforcement learning task

Authors: *N. F. PARKER[1], T. J. DAVIDSON[2], C. M. CAMERON[1], J. P. TALIAFERRO[1], N. D. DAW[3], I. B. WITTEN[1];
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Abstract: Previous research has shown dopamine (DA) neurons that project to the striatum play an important role in reinforcement learning by encoding the difference between the expected and actual reward (i.e. prediction error). However, different sub-populations of dopamine neurons project to different striatal subregions. If, and how, the function of these sub-populations differs remains an open question. To investigate this question, we sought to measure neural activity in DA terminals in the dorsal and ventral striatum during a probabilistic reward learning task. We first validated that the task required DA activity by transiently inhibiting activity in DA cell bodies on a randomly selected subset of trials and observing disruptions in trial-by-trial learning. We then measured DA activity in terminal regions in the striatum using gCaMP6f, and developed a statistical model to dissociate the contribution of each task event to the DA signal in each striatal region. We found that DA terminals in these two regions show markedly different responses. For example, dopamine neurons projecting to ventral, but not dorsal, striatum respond to rewards even when they are predicted. These results further illuminate our understanding of the function of DA, a molecule involved in a range of disorders, as well as our general understanding of how the dynamics in neuromodulatory neurons can contribute to learning.

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Poster

723. Appetitive and Incentive Learning and Memory II

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.13/AA24

Topic: F.02. Animal Cognition and Behavior

Support: CONACYT CB-2012/177624

Title: The long term consumption of hypocaloric diets poor in vitamin c and protein exposed to temperatures from 40 to 50°c alters anxiety behavior

Authors: *R. B. GARCIA[1], A. E. GÓMEZ-MARTÍNEZ[2], T. NERI-GÓMEZ[3], T. C. SOSA-LARIOS[4], A. CHINCHILLAS-SÁNCHEZ[5], A. C. MÉNDOZA-REYES[6], S. L. MORIMOTO-MARTÍNEZ[7];
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Mexico; [6]BIOLOGY, UNAM, MEXICO CITY, Mexico; [7]BIOLOGÍA DE LA REPRODUCCIÓN, INSITUTUTO NACIONAL DE CIENCIAS MEDICAS Y NUTRICIÓN, MEXICO CITY, Mexico

Abstract: Food is vital to human existence, and requires special care like suitable storage temperatures to increase shelf life, which leads to maintaining their nutritional qualities. However when storage conditions are not adequate, as in the case of an excessive temperature rise, this can affect its nutritional content and when the food is ingested may not provide recommended amounts of daily consumption nutrient or nutrients such as: carbohydrates, proteins, minerals, vitamins, fat and water; causing malnutrition that can lead not only physiological but also behavioral disorders, such as anxiety, depression, obsessive compulsive disorders or impaired memory. The aim for this study was to evaluate different diets and how can alter anxiety behavior. For this purpose Wistar rats (45 ± 5 g) were used; animals were divided into 4 groups (n=6 per group), and were placed in a rack individually, with the following diets: A = Reference diet, B = Control restricted diet, the following groups were given a hypocaloric diet with low vitamin C and protein exposed to different temperatures; C= 40°C, and D = 50°C, daily feed intake per rat and body weight were weighted for 30 days with free access to water and maintained under conditions indicated in the NOM 062 -ZOO-1999 and with international specifications for the production, care and use of laboratory animals standards. After 45 days with the diets, rats were evaluated with the anxiety test using the marble burying model for 10 min, and black and white model for 10 minutes, likewise the locomotors activity assessment was performed for 5 min. Data were collected and analyzed using one-way ANOVA, post-hoc test: Duncan's for significant differences with $p < 0.05$ (sigma stat program version 3.2). Anxiogenic effect and modification of biochemical parameters (Glucose, total cholesterol and triglycerides) and insulin secretion of insulin were decreased in groups C and D compared to controls. Our data suggest that and hypocaloric diet and an augment of temperature can increase the anxiety and alter the biochemical parameters involved in the control of metabolism.

Disclosures: **R.B. Garcia:** A. Employment/Salary (full or part-time):; UNAM, FACULTAD DE QUIMICA. 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.; INSTITUTO NACIONAL DE CIENCIAS MEDICAS Y NUTRICIÓN. **A.E. Gómez-Martínez:** A. Employment/Salary (full or part-time):; UNAM, FACULTAD DE QUIMICA. **T. Neri-Gómez:** None. **T.C. Sosa-Larios:** None. **A. Chinchillas-Sánchez:** None. **A.C. Méndoza-Reyes:** None. **S.L. Morimoto-Martínez:** 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.; INSTITUTO NACIONAL DE CIENCIAS MEDICAS Y NUTRICIÓN "SALVADOR ZUBIRAN".

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.14/AA25

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA016285-03

Title: Systemic dopamine D1 and D2 receptor agonism differentially reverses environmental enrichment attenuated sucrose cue-reactivity after 1 or 30 days of forced abstinence

Authors: E. GLUECK, D. GINDER, J. HYDE, F. GRIFFIN, N. INGERMANN, K. NORTH, H. REISTERER, M. KROLL, *J. W. GRIMM;
Dept of Psych and Beh Neurosci, Western Washington Univ., Bellingham, WA

Abstract: Exposure to either acute or chronic environmental enrichment (EE) profoundly reduces sucrose cue-reactivity by rats. The present experiment was conducted to examine whether dopamine D1 or D2 receptor agonism could reverse these effects. The experiment also included rats tested for cue-reactivity after either 1 or 30 days of forced abstinence to examine whether drug effects would vary with the incubation of sucrose craving. METHODS: Effects of systemic dopamine D1 (SKF-81297) or D2 (quinpirole) agonism were examined following 10 d (2h/d) of sucrose self-administration and a 2h cue-reactivity test on a subsequent day (n=10-11 per group). Prior to this test, rats experienced either 1 or 30 d of forced abstinence and either overnight (acute) or 29 d (chronic) EE. Following each housing manipulation, and immediately prior to cue-reactivity testing, rats received a systemic injection of either D1 agonist (0, 0.3, or 1 mg/kg) or D2 agonist (0, 0.1, or 0.3 mg/kg). EE consisted of 3 rats housed in a large, multi-level cage with novel toys exchanged 3 times a week. Controls were returned to single housing. RESULTS: High doses of D1 or D2 agonist increased day 1 acute EE cue-reactivity compared to low dose and vehicle injected animals. This level of responding was comparable to control animals. Neither agonist affected responding of control rats. On the day 30 test, both doses of D1 agonist increased cue-reactivity in acute EE, chronic EE, and control rats. Further analysis revealed that both doses significantly increased responding of chronic EE rats above vehicle treated controls. In contrast, both doses of D2 agonist decreased lever responding of control housed rats while the high dose slightly, but not significantly, increased responding of Day 30 acute EE rats ($p=0.05$). CONCLUSION: Both D1 and D2 receptors are involved in the acute EE mediated decrease in sucrose cue-reactivity observed following one day of forced abstinence from sucrose self-administration. In contrast, following 30 days of forced abstinence D1 receptors may be especially critical in cue-reactivity as D1 agonist was effective at both restoring responding of enriched animals and potentiating responding of controls. This may indicate an

interaction between EE and incubation of craving effects specific to the D1 receptor. Furthermore, the diminished responding of controls following D2 agonist after 30 days of forced abstinence may indicate a change in D2 sensitivity (either a leftward or rightward shift) specific to the incubation of craving.

Disclosures: **E. Glueck:** None. **D. Ginder:** None. **J. Hyde:** None. **F. Griffin:** None. **N. Ingermann:** None. **K. North:** None. **H. Reisterer:** None. **M. Kroll:** None. **J.W. Grimm:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.15/AA26

Topic: F.02. Animal Cognition and Behavior

Support: JSPS KAKENHI Grant Number 24530930

Title: Environmental enrichment attenuates incubation of cue-induced reinstatement of sucrose seeking but not sucrose consumption in rats

Authors: *K. AOYAMA;
Doshisha Univ., Kyotabnabe, Japan

Abstract: Environmental enrichment (EE) reduces drug and sucrose cue-reactivity in rats. In addition, a previous study (Grimm, Weber, Barnes, Koerber, Dorsey, & Glueck, 2013) reported that EE attenuated sucrose consumption. In the previous study, the sucrose consumption test was conducted on the day after the cue-reactivity test in which lever-press responses were not followed by sucrose presentation. Therefore, interpretation of the consumption test was difficult because the lever-press response may be extinguished during the cue-reactivity test. The present study reversed the order of the two tests. Twenty-eight rats were trained to press a lever for sucrose pellets in 10 daily sessions (self-administration training). Sucrose delivery was accompanied by a tone+light cue. Rats were then divided into three groups. In the Day 1 group, the consumption test was conducted on the day after the last day of the self-administration training (Day 1). The cue-reactivity test was conducted on the next day (Day 2). In the Day 30 and EE groups, the consumption test was conducted after 30 days of forced abstinence of sucrose (Day 30) and the cue-reactivity test was conducted on the next day (Day 31). The procedure of the consumption test was identical to the self-administration training. In the cue-reactivity test, each lever-press was followed by the tone+light cue but not by a sucrose pellet. After the self-administration training, sucrose was not provided until the consumption test. During the forced

abstinence phase, rats in the EE group were placed in larger cages with novel objects and were housed in pairs. In both tests, rats in the Day 30 group responded more than rats in the Day 1 group, indicating the incubation of sucrose craving. Rats in the EE group responded as much as rats in the Day 30 group in the consumption test, but they responded less than rats in the Day 30 group in the cue-reactivity test. The results suggest that EE attenuates incubation of sucrose seeking regardless of the order of the two tests, but the effect of EE on incubation of sucrose consumption depends on the order of the two tests. These results may be useful in the development of anti-relapse strategies for drug and food addictions.

Disclosures: K. Aoyama: None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.16/AA27

Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIDA R15 DA035432

Title: Chronic restraint stress causes a delayed increase in craving for palatable food via a dopamine D_1-like receptor-mediated mechanism

Authors: *K. T. BALL, O. BEST, J. LUO, L. MILLER;
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Abstract: Evidence from the clinical literature suggests that relapse to unhealthy eating habits in dieters is often triggered by acute exposure to stress, palatable food, or food-associated cues. Using an animal model of relapse, we reported recently that chronic exposure to the pharmacological stressor yohimbine following extinction of food seeking potentiated later reinstatement of food seeking induced by acute yohimbine. Furthermore, SCH-23390, a dopamine D_1-like receptor antagonist, combined with repeated yohimbine injections reversed the effect of yohimbine on later reinstatement. To extend these findings, we tested the effect of chronic restraint stress on responding for food-associated cues during abstinence. Thus, male, Sprague-Dawley rats were trained to press a lever for highly palatable food reinforcers (12.7% fat, 66.7% carbohydrate, and 20.6% protein) in daily 3-hr sessions. Subsequently, rats were tested for food seeking both before and after a chronic restraint stress procedure (3 h/day x 10 days) or control procedure (unstressed). To assess dopaminergic involvement, each group received daily injections of either SCH-23390 (10.0 μg/kg; i.p.) or vehicle prior to treatment. To

examine time-dependent effects of stress, the second food seeking test was conducted either 1 day or 7 days after the last restraint. Results showed that vehicle-injected stressed rats displayed increased responding 7 days, but not 1 day, after the last restraint. Moreover, SCH-23390 combined with stress reversed this effect. These data suggest that chronic stress induces neural changes via a dopamine D_1-like receptor mediated mechanism that are expressed behaviorally as increased food seeking after a stress-free period. From a clinical perspective, our findings suggest that individuals on a dietary treatment plan who also are exposed to chronic stress may be most vulnerable to cue-induced craving and relapse at some point *after* the chronic stress has subsided. In addition, treatment with drugs targeting D_1-like receptors during chronic stress may help to prevent future cue-induced relapse.

Disclosures: **K.T. Ball:** None. **O. Best:** None. **J. Luo:** None. **L. Miller:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.17/AA28

Topic: F.02. Animal Cognition and Behavior

Support: DGAPA-PAPIIT IN209911

CONACyT 152208

Title: Changes on prefrontal dopaminergic receptors, appetitive taste memory retrieval and appetitive re-learning, induced by prolonged sugar consumption

Authors: *S. CAYNAS[1], G. RODRÍGUEZ-GARCÍA[1], I. DELINT-RAMÍREZ[2], M. I. MIRANDA[1];
[1]Univ. Nacional Autonoma de Mexico - Inst. de Neurobiologia, Querétaro, México, Mexico;
[2]Univ. Autonoma De Nuevo Leon, Monterrey, Mexico

Abstract: The medial prefrontal cortex (mPFC) is implicated in a variety of cognitive functions that are important for a maladaptive appetitive behavior, such as habit formation and appetitive learning. Within mPFC, the dopamine neurotransmitter system plays a key role in appetitive learning; nevertheless, it is not known if overtraining appetitive learning compromises the dopamine system in the mPFC and how these changes promote compulsive consumption despite the negative consequences. In the present work we evaluated the effect of apomorphine, a dopaminergic agonist and haloperidol an antagonist of dopamine receptors in the mPFC on the

acquisition and extinction of a new aversive association with a taste that was previously exposure either in an acute (sugared water at 10% for 20 min daily on 3 days; familiar group; F-group) or prolonged fashion (sugared water at 10% for 21 days ad libitum; high familiar group (HF-group). The result showed that HF-group had weaker memory trace of the new aversive association compared to the F-group. Also, we found that prefrontal dopamine receptors blockade in the HF-group prevents high familiar appetitive taste memory retrieval and appetitive re-learning (i.e., extinction of aversion), whereas both activation and blockade of dopamine receptors in the mPFC disrupt the new aversive association in the F-group. Furthermore, using Western Blot, we found a greater amount of prefrontal extrasynaptic receptors in the HF-group. Altogether these results suggest an increase of prefrontal dopaminergic transmission which underlies high familiar appetitive taste memory recognition and could mediate the shifting from taste preference to taste aversion.

Disclosures: **S. Caynas:** None. **G. Rodríguez-García:** None. **I. Delint-Ramírez:** None. **M.I. Miranda:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.18/AA29

Topic: F.02. Animal Cognition and Behavior

Title: Escalation of palatable food consumption by rats with increased reward availability

Authors: *S. T. WHITE[1], I. KRASNOVA[2], J. L. CADET[2];
[1]Pharmacol., [2]Neuropsychiatry research Br., Natl. Inst. on drug abuse, Baltimore, MD

Abstract: Chronic compulsive overeating or food addiction can lead to loss of control over food consumption despite negative medical consequences. In this study, we used a rat model of palatable food self-administration in order to investigate if rats will escalate their palatable food intake and gain excessive weight. Male rats were trained to self-administer palatable food rewards for 3 separate sessions of 3-hour each separated by a 30 min break (total of 9 hours per day) for 20 days. Reward delivery was paired with a tone-light cue. After one week of palatable food reward administration, animals were divided into two subgroups that consisted of (1) stable reward (SR) and (2) reward escalation (RE) groups. The SR group received a maximum of 35 rewards throughout the entire study whereas the RE group was allowed weekly increases in the number of rewards. Subsequently, we assessed cue-induced reward seeking in 1-h extinction sessions on withdrawal days 2 and 21. We found that the RE group pressed the lever

significantly more and received greater number of rewards than the SR group. Both groups consumed most of the rewards within the first hour of each 3-hour session. The RE but not the SR group showed greater weight gains than control animals. Interestingly, both the SR and the RE group showed comparable cue-induced lever presses at day-2 and day-21 of extinction testing, with the RE group showing higher number of lever presses than the SR group. These results suggest a higher level of craving in the RE group. Further research is necessary to identify potential molecular differences that may account for the behavioral differences between the two groups.

Disclosures: S.T. White: None. **I. Krasnova:** None. **J.L. Cadet:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.19/AA30

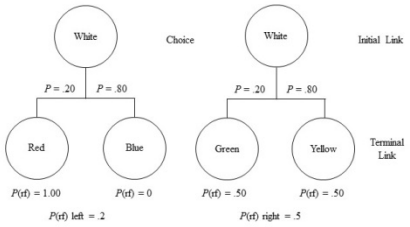
Topic: F.02. Animal Cognition and Behavior

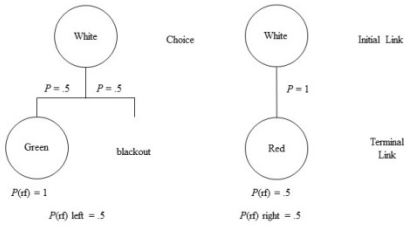
Title: Incentive salience attributed to a reward-associated stimulus promotes suboptimal choice in pigeons and rats

Authors: *A. P. SMITH, J. J. CHOW, A. BAILEY, T. R. ZENTALL, J. S. BECKMANN; Psychology, Univ. of Kentucky, Lexington, KY

Abstract: Previous research has shown pigeons reliably choose a suboptimal alternative associated with less food. In these instances, pigeons choose between two initial link stimuli where choice on the suboptimal alternative had a 20 percent probability of producing a stimulus that guarantees food or an 80 percent probability of receiving a stimulus that signals reward omission; the optimal alternative produced one of two stimuli (with 20 and 80 percent frequencies) that each predicted food 50 percent of the time. This effect appears driven by pigeons' overweighting of the more predictive conditioned reinforcer (stimulus that guarantees food) and not taking into account its frequency (20 percent) or the more frequent (80 percent) outcome of omission. The present experiments focused on examining how different conditioned reinforcers in an analogous task can influence choice across species. Pigeons and rats were given a choice between two initial links for a predictive and non-predictive alternative. A predictive choice had equal probability of producing a stimulus predicting food or a blackout period (non-reinforcement). Alternatively, a non-predictive choice was always followed by a stimulus that predicted food 50 percent of the time. In this phase, where food opportunities were equal between the two alternatives, choices in both species showed strong preference for the predictive

stimulus. Subsequent phases reduced the frequency of the predictive stimulus and food exponentially (from 50 percent to 25, 12.5, and 0) resulting in maintained preferences despite less food yield until close to extinction. Thus, maladaptive choice in both pigeons and rats appears to be driven by the value attributed to stimuli that predict reinforcement even if they result in fewer reinforcers. Additionally, this decision making process in gambling-like risky choice behavior shares parallels in how reward-associated stimuli are linked to substance abuse.





Disclosures: **A.P. Smith:** None. **J.J. Chow:** None. **A. Bailey:** None. **T.R. Zentall:** None. **J.S. Beckmann:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

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Program#/Poster#: 723.20/AA31

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant K99-MH105549

NIH Grant P50-MH086400

Title: The paraventricular nucleus of the thalamus regulates cued food-seeking during reward omission

Authors: *F. H. DO MONTE, A. M. MINIER-TORIBIO, G. J. QUIRK;
Psychiatry and Anat. & Neurobio., Univ. of Puerto Rico, San Juan, PR

Abstract: Recent studies have shown that the paraventricular nucleus of the thalamus (PVT) is a key region for retrieval of well-consolidated fear memories. Particularly, silencing of PVT projections to the central nucleus of the amygdala (CeA) impairs both retrieval and maintenance of cue-associated fear memories (Do Monte et al, 2015; Penzo et al 2015). Studies have also implicated PVT in cue-associated reward memories (Haight and Flagel, 2014; Matzeu et al., 2014). Here, we sought to understand the role of PVT and its projections in cued food-seeking. We used a reward conditioning task in which rats learned that each bar press in the presence of a light cue delivered a sugar pellet into a nearby dish. We therefore manipulated PVT activity under two conditions: 1) when food was available during the cue (positive outcome), or 2) when food was omitted during the cue (negative outcome). Inactivation of the anterior PVT, but not the posterior PVT, with fluorescently labeled muscimol increased pressing when food was omitted (aPVT Sal: 26.6 presses/min, n= 9, aPVT Mus: 40.8 presses/min, n= 5; p= 0.015). To assess the role of PVT projections to CeA during positive vs. negative outcomes, we used an optogenetic approach (halorhodopsin, AAV5:CaMKIIa::eNpHR3.0-eYFP) to specifically silence PVT terminals in CeA during the cue presentation. Silencing of PVT-CeA projections reduced pressing during the cue when the food was omitted (eYFP-Control: 21 presses/min, n= 8, NpHR-eYFP: 13.1 presses/min, n= 8; p< 0.001), but not when the food was available (p= 0.88). Our findings suggest that distinct antero-posterior subregions of PVT regulate food-seeking during negative states induced by food omission. We are currently examining PVT projections to nucleus accumbens, a region involved in reward.

Disclosures: F.H. Do Monte: None. **A.M. Minier-Toribio:** None. **G.J. Quirk:** None.

Poster

723. Appetitive and Incentive Learning and Memory II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 723.21/AA32

Topic: F.02. Animal Cognition and Behavior

Support: NSERC RGPIN 402642

Title: Approach-avoidance processing: the role of nucleus accumbens shell D1 and D2 receptors in conflict resolution

Authors: *D. NGUYEN, V. FUGARIU, R. ITO;
Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: The nucleus accumbens (NAc) is importantly implicated in the processing of approach and avoidance signals evoked by emotionally valenced environmental stimuli. Currently, there is evidence to suggest that such opposing motivational processes are differentially mediated by subpopulations of NAc neurons expressing either dopaminergic D1- or D2-receptors (D1R, D2R). Further, it has been suggested that dysregulation of this system is implicated in disorders such as drug addiction, where the individual elicits aberrant processing of competing motivational signals. It is therefore important to further elucidate the mechanisms that mediate approach-avoidance processing in states of motivational conflict. The present study utilized a mix-valenced conditioning paradigm to examine the effects of NAc shell D1R or D2R antagonism on approach-avoidance behavior. Male Long Evans rats were trained in a three-arm radial maze to associate visuo-tactile cues with sucrose, shock, or neutral outcomes delivered within the arms in which the cues were presented. Following conditioning, rats were intracerebrally infused with D1R antagonist SCH23390 or D2R antagonist Sulpiride in the NAc shell. Exploration time was then assessed in a conflict test where rats freely explored two maze arms containing either a neutral cue or a superimposition of the appetitive and aversive cues under extinction conditions. Our results revealed that D2R antagonism enhanced preference for the mix-valenced arm, while D1R antagonism appears to decrease preference for the mix-valenced arm, albeit our D1R data is currently preliminary. We conclude that NAc shell D2R is important for suppressing approach behaviors, and NAc shell D1R may be importantly implicated in eliciting approach behaviors, when the valence of the outcome is uncertain.

Disclosures: **D. Nguyen:** None. **V. Fugariu:** None. **R. Ito:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

Location: Hall A

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Program#/Poster#: 724.01/AA33

Topic: F.02. Animal Cognition and Behavior

Support: This work was supported by a German Research Foundation (DFG) grant to DMV (SFB874, B1).

Title: Investigations of the human cortical networks involved in task-irrelevant spatial change detection

Authors: *M. F. HAUSER[1,2], V. WIESCHOLLECK[2], C. BELLEBAUM[3], D. MANAHAN-VAUGHAN[1,2];
[1]Ruhr Univ. Bochum, Bochum, Germany; [2]Intl. Grad. Sch. of Neurosci., Bochum, Germany; [3]Inst. of Exptl. Psychology, Heinrich Heine Univ., Düsseldorf, Germany

Abstract: The hippocampus, as a key structure for the formation of spatial memory, responds to, and integrates, novel information into existing frameworks. However, studies as to whether it is sensitive to novelty on an environmental level, or whether it responds primarily to content-novelty have produced conflicting results. Electrophysiological experiments in rodents suggest that the hippocampus supports both, integration of novel context as well as content, by means of synaptic plasticity (Manahan-Vaughan & Braunewell PNAS 1999, 96:8739; Kemp & Manahan-Vaughan, Trends Neurosci. 2007, 30:111). Furthermore, hippocampal long-term depression (LTD) plays a role in the processing of object novelty, as well as spatial content, even when passively viewed (Kemp & Manahan-Vaughan, PNAS, 2004,101:8192; Cereb Cortex, 2012, 22:1614). As a corollary to the abovementioned rodent studies, we conducted a human event-related potential (ERP)-study that explored passive viewing of reconfigurations of familiar abstract objects. Participants implemented a distracter-task while background images were presented akin to an oddball paradigm. The deviant condition depicted a spatially reconfigured version of the standard image. This spatial change exclusively elicited positivities in parieto-occipital electrodes at around 400ms, which after analysis using sLORETA, were traced to left inferior occipital areas. Furthermore, at around 500ms, a temporal negativity similar to that observed for novel stimuli was found. Interestingly, when images were viewed for a 2nd time in direct succession, this temporal effect was no longer evident, speaking for an immediate integration and storing of novel or changed information. Taken together, our data support that perception of task-irrelevant spatial information leads to temporal processing, in a way suggestive of memory-updating.

Disclosures: M.F. Hauser: None. **V. Wieschollleck:** None. **C. Bellebaum:** None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 724.02/AA34

Topic: F.02. Animal Cognition and Behavior

Support: German Research foundation (DFG), SFB874/B1.

Title: The metabotropic glutamate receptor mGlu5 regulates the direction of opposing forms of synaptic plasticity at mossy fiber - CA3 and commissural/associational - CA3 synapses

Authors: *D. MANAHAN-VAUGHAN, H. HAGENA;
Ruhr Univ. Bochum,, Bochum, Germany

Abstract: The direction of synaptic plasticity, expressed as long-term potentiation (LTP) and long-term depression (LTD) within the hippocampus is affected by spatial experience. The CA3 region plays a pivotal role in the processing of spatial information and hence spatial experience. CA3 receives two inputs formed by mossy fibers (MF) and associational/commissural fibers (AC) that are believed to help in engaging working memory, pattern separation and pattern completion. MF-CA3 and AC-CA3 synapses both express LTP and LTD, but it is unclear how information processing is regulated at these two synapses. We speculated that the metabotropic glutamate receptor, mGlu5, could be a key player, given its contribution in enabling synaptic plasticity in other hippocampal subfields. LTD and LTP were examined at MF-CA3 and AC-CA3 synapses of freely behaving adult rats. The mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl) pyridine (MPEP) inhibited LTP at MF-CA3 synapses, but not at AC-CA3 synapses. By contrast, mGlu5 antagonism prevented LTD at AC-CA3 synapses but not at MF-CA3 synapses. This suggests that activation of mGlu5 preferentially leads to LTP at MF-synapses, whereas LTD is promoted at AC-synapses. We verified this possibility by means of synaptic stimulation at 50 Hz. This frequency corresponds to Theta-m (Bienenstock et al., J Neurosci 1982, 2:32) that corresponds to the crossover-point for mechanisms that enable LTD and LTP at CA3 synapses. 50 Hz stimulation in the presence of the mGlu5 receptor agonist (R,S)-2- chloro-5-hydroxyphenylglycine (CHPG) resulted in a synaptic potentiation of MF-CA3, but not of AC-CA3 responses. These findings indicate that the mGlu5 receptor may act as a switch that alters signal-to-noise ratios during information encoding and thereby supports highly specific storage of information in CA3 by means of LTP and LTD.

Disclosures: D. Manahan-Vaughan: None. **H. Hagena:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 724.03/AA35

Topic: F.02. Animal Cognition and Behavior

Support: German Research foundation (DFG), SFB874/B1

Title: Engagement of the descending and ascending pathways to the piriform cortex in synaptic plasticity in behaving rodents

Authors: *C. STRAUCH[1,2], D. MANAHAN-VAUGHAN[1,2];
[1]Dept. of Neurophysiol., Ruhr-University Bochum, Med. Fac., Bochum, Germany; [2]Intl. Grad. Sch. of Neurosci., Ruhr-University Bochum, Germany

Abstract: It is known that all regions of the cortex can undergo experience-dependent modifications. The primary visual cortex for example engages in pre-processing of visual information in the form of persistent synaptic plasticity (Tsanov and Manahan-Vaughan 2007, J. Neurosci). But so far, it is not clear if this property is shared or emulated by other sensory cortices. The olfactory system contrasts strongly with all other sensory systems, because primary and higher cortical regions are involved in information processing before the thalamus is engaged. Early *in vivo* studies reported that repetitive afferent high-frequency stimulation of the olfactory bulb cannot induce synaptic plasticity in the olfactory (piriform) cortex (Racine et al. 1983, Brain Res; Stripling et al. 1988, Brain Res). Even so *in vitro* studies revealed that afferent stimulation can evoke potentiation (Kanter and Haberly 1990, Brain Res), but very rarely (Jung et al. 1990, Synapse). We explored whether synaptic plasticity can be induced in the piriform cortex by patterned afferent stimulation. Animals were chronically implanted with a recording electrode in the anterior piriform cortex, and a bipolar stimulation electrode was implanted into the olfactory bulb or the orbitofrontal cortex. Patterned afferent stimulation protocols at various frequencies (0.5-400 Hz) were applied to the olfactory bulb or the orbitofrontal cortex and evoked responses were examined in the anterior piriform cortex in behaving animals. In the ascending pathway from the olfactory bulb, no synaptic plasticity in the piriform cortex was elicited regardless of the afferent stimulation protocol used. In contrast, after high-frequency stimulation of the orbitofrontal cortex (descending pathway) long-term potentiation in the piriform cortex was evoked. These data suggest that in the ascending pathway to the piriform cortex no synaptic plasticity can be induced in behaving animals. Furthermore, top-down control, for example from the orbitofrontal cortex, might control synaptic plasticity in the piriform cortex. This work was supported by a German Research Foundation (DFG) grant to DMV (SFB874, B1)

Disclosures: C. Strauch: None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 724.04/AA36

Topic: F.02. Animal Cognition and Behavior

Support: German Research foundation (DFG), SFB874/B1

Title: Hippocampal synaptic information encoding in the form of LTD is enable by olfactospatial and audiospatial learning

Authors: *B. E. DIETZ[1,2], M. A. E. ANDRÉ[1,2], D. MANAHAN-VAUGHAN[1,2];
[1]Dept. of Neurophysiol., Ruhr Univ. Bochum, Med. Fac., Bochum, Germany; [2]Intl. Grad. Sch. of Neurosci., Ruhr-University Bochum, Germany

Abstract: Hippocampal long-term depression (LTD) is intrinsically involved in the encoding of novel spatial experience (Manahan-Vaughan & Braunewell, PNAS, 1999, 96:8739; Kemp & Manahan-Vaughan, Trends Neurosci., 2007, 30:111). Hippocampal LTD is especially associated with the acquisition of information about spatial content (Kemp & Manahan-Vaughan, PNAS, 2004,101:8192; Cereb Cortex, 2012, 22:1614). It is unclear to what extent spatial information derived from other sensory modalities can enable information encoding by means of LTD. Here, we explored whether novel olfactospatial, or audiospatial learning facilitates hippocampal LTD. We observed that novel spatial configurations of discretely arranged olfactory, or auditory, stimuli facilitated the expression of hippocampal LTD in freely behaving adult rats. In both cases, LTD was enable by novel spatial learning. Strikingly, audiospatial effects were more potent, and could be better differentiated by the animals when behaviorally relevant tones were used. The resultant LTD endured for at least 4h for both olfactospatial and audiospatial cues, and was tightly coupled to the learning event. Taken together with previous observations with regard to visuospatial information processing, these data suggest that for the hippocampus the sensory modality is not crucial for the enablement of synaptic information encoding. Rather it is the saliency and behavioral relevance of the modality that is decisive in triggering synaptic storage of the spatial experience. This work was supported by a German Research Foundation (DFG) grant to DMV (SFB874, B1)

Disclosures: **B.E. Dietz:** None. **M.A.E. André:** None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

Location: Hall A

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Program#/Poster#: 724.05/AA37

Topic: F.02. Animal Cognition and Behavior

Support: EU 7th Framework program grant HEALTH-F2-2007-201159

Title: Acute intracerebral treatment with amyloid-beta (1-42) modifies neuronal oscillations that occur in conjunction with LTP induction and impairs LTP *in vivo*

Authors: *A. N. KALWEIT[1], H. YANG[1], J. COLITTI-KLAUSNITZER[1], Z. BOZSÓ[2], L. FÜLÖP[2], B. PENKE[2], D. MANAHAN-VAUGHAN[1];
[1]Med. Faculty, Dept. Neurophysiol., Ruhr-University Bochum, Bochum, Germany; [2]Univ. of Szeged, Szeged, Hungary

Abstract: Aβ-oligomers play a crucial role in the pathophysiology of Alzheimer´s disease. Acute treatment with human or synthetic Aβ(1-42) impairs hippocampal long-term potentiation (LTP), a cellular mechanism that is believed to contribute to hippocampus-dependent learning. Along with changes in somatic and dendritic field potentials, theta-gamma oscillations that occur during high-frequency stimulation (HFS) to induce LTP predict whether successful LTP will occur (Bikbaev et al, 2008, Front Neurosci. 2:56-63). In this study, we explored to what extent changes in neuronal oscillations accompany deficits in LTP that occur as a result of acute Aβ(1-42) treatment. Stable LTP that lasted for over 24h was expressed in 6-month-old rats. Acute treatment with Aβ prevented LTP in these animals. During HFS of control animals relative theta power was reduced in control animals, whereas in Aβ-treated animals this suppression of theta power was absent. Aβ-treated animals also exhibited a lower amount of envelope-to-signal (ESC) correlations between hippocampal theta amplitudes and fast gamma envelopes. Furthermore, during HFS of healthy animals, a significant correlation between ESC-scores, relative theta and gamma power was apparent, that was not evident Aβ-treated animals. These data indicate that Aβ(1-42) causes deficits in information processing at the level of neuronal oscillations, that occur in conjunction with impaired synaptic plasticity. These impairments are likely to contribute to the loss of hippocampus-dependent memory storage ability that occurs in Alzheimer's disease.

Disclosures: **A.N. Kalweit:** None. **H. Yang:** None. **J. Colitti-Klausnitzer:** None. **Z. Bozsó:** None. **L. Fülöp:** None. **B. Penke:** None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 724.06/AA38

Topic: F.02. Animal Cognition and Behavior

Support: German Research foundation (DFG), SFB874/B3

Title: Influence of directional information in place field representations of space

Authors: *M. LORKOWSKI[1,2], S. ZHANG[1,2], F. DRAHT[1,2], F. SCHÖNFELD[3,2], L. WISKOTT[3,2], D. MANAHAN-VAUGHAN[1,2];
[1]Med. Faculty, Dept. Neurophysiol., [2]Intl. Grad. Sch. of Neurosci., [3]Inst. for Neural Computation, Ruhr Univ. Bochum, Bochum, Germany

Abstract: The ability of the hippocampus to reliably encode and navigate space critically relies on the integration of a variety of different input sources. Spatial representation is heavily dependent on sensory information that generates a spatial context for navigation. This predominantly derives from visual information. However, spatial navigation is also supported by internal information from e.g. the vestibular or proprioceptive systems that enable path integration (Zhang & Manahan-Vaughan, 2013). To what extent path integration, and more particularly directional information, can serve as a contextual cue for place cell activity is unclear. To elucidate this we recorded place cells in the CA1 area of the hippocampus using a behavioral paradigm that was specifically designed to create a conflict between sensory and directional information (derived from idiothetic cues). Animals (male Long-Evans rats) navigated different L-shaped routes towards two identical chambers in white noise and darkness (context A). Subsequently, the L-shaped routes were merged so that the animal could turn left or right from the access corridor, to access either chamber (context B). In context A, we observed that place fields emerged in identical locations in the 2 chambers. In context B, place fields initially did not remap, and were thus controlled by proximal visual cues that override directional information, when both are in conflict. However, upon repeated exposure to context B, remapping occurred, suggesting that information provided by directional cues became the primary determinant in driving place cell activity.

Disclosures: **M. Lorkowski:** None. **S. Zhang:** None. **F. Draht:** None. **F. Schönfeld:** None. **L. Wiskott:** None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Program#/Poster#: 724.07/AA39

Topic: F.02. Animal Cognition and Behavior

Support: German Research Foundation (DFG) grant to DMV (FOR1581, P2)

Title: Role of BDNF in context-dependent and independent extinction of an appetitive spatial learning task

Authors: *A. B. LEHR[1,2], M. A. E. ANDRÉ[1,2], D. MANAHAN-VAUGHAN[1,2];
[1]Med. Faculty, Dept of Neurophysiol., [2]Intl. Grad. Sch. of Neurosci., Ruhr Univ. Bochum, Bochum, Germany

Abstract: The process of suppressing a previously acquired behavior when it is no longer useful is referred to as extinction learning. Brain-derived neurotrophic factor (BDNF) is required for mechanisms that underlie learning and memory and supports hippocampal synaptic plasticity (Novkovic and Manahan-Vaughan, 2015, Hippocampus 25:1). Recently, it has emerged that the hippocampus is also likely to contribute to context-dependent extinction learning, whereby to-date much attention has been paid to extinction of aversive learning forms. Here, we explored the role of BDNF in context-dependent and context-independent appetitive extinction learning in rodents. Animals were trained to learn that food could be found (with low probability) at the end of one arm of a T-maze (context A). Following successful learning, the floor, distal cues and odor-context of the maze were changed and extinction learning under unrewarded conditions was examined (context B) (ABA paradigm). Reactivation/renewal of the original learned behavior was tested one day later in the unrewarded A context. In a second group, the context remained constant throughout all trials (AAA paradigm). In BDNF+/- mice tested in the ABA paradigm, extinction learning in the B context was impaired, and renewal behavior in the A context was stronger than in wildtype littermates. In BDNF+/- mice tested in the AAA paradigm, extinction in the A context was unaffected. These data suggest that BDNF contributes to context-dependent extinction. This may relate to hippocampal processes supported by BDNF, and the particular role played by the hippocampus in context-dependent learning.

Disclosures: **A.B. Lehr:** None. **M.A.E. André:** None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

Location: Hall A

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Program#/Poster#: 724.08/AA40

Topic: F.02. Animal Cognition and Behavior

Support: German Research Foundation (DFG) grant to DMV (SFB874, B10)

Title: BDNF knock-down lead to impairments of hippocampal synaptic plasticity *in vivo* and contributes to the facilitation of synaptic plasticity through environmental enrichment

Authors: *J. AARSE[1,2], T. NOVKOVIC[1,2], D. MANAHAN-VAUGHAN[1,2];
[1]Dept. Neurophysiol., Ruhr Univ. Bochum, Med. Fac., Bochum, Germany; [2]Intl. Grad. Sch. of Neurosci., Bochum, Germany

Abstract: Brain-derived neurotrophic factor (BDNF) supports a multitude of brain processes that are required for effective cognition, such as synaptogenesis, neurogenesis, and mechanisms that underlie learning and memory. Persistent synaptic plasticity is believed to underlie long-term information storage, but to what extent BDNF is required for forms of plasticity that last for hours or days is unclear. Environment enrichment (EE) enhances hippocampal synaptic plasticity, and alters BDNF expression. Here, we explored the role of BDNF in persistent forms of hippocampal plasticity under naïve and enriched environmental conditions in transgenic mice that underwent a partial knockout of BDNF. In hippocampal slices *in vitro*, wildtype mice (WT) expressed long-term potentiation (LTP) in the CA1 region that lasted for at least 60 min. LTP was impaired in BDNF+/- mice. In freely behaving mice, LTP elicited by high-frequency stimulation (100Hz) was equivalent in WT and heterozygous BDNF+/- siblings, and lasted for over 24h. Afferent stimulation using a theta-burst protocol (TBS) elicited LTP in WT and revealed impaired LTP in transgenics. Short-term and long-term depression (LTD) was impaired both *in vitro* and *in vivo* in BDNF+/--mice. BDNF+/--mice exhibited deficits in spatial memory. EE improved LTP and object recognition memory in WT and BDNF+/--mice, whereby LTP in transgenics achieving levels seen in wildtypes in the absence of EE. EE resulted in increased levels of BDNF, but not proBDNF. These findings indicate that BDNF plays an intrinsic role in persistent forms of hippocampal synaptic plasticity, and that it is an important component in the cellular mechanisms that underlie optimal cognition.

Disclosures: J. Aarse: None. **T. Novkovic:** None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: (DFG) SFB874, B1

Title: Impaired hippocampal function as a consequence of psychosis

Authors: *V. DUBOVYK, T. GRÜTER, V. WIESCHOLLECK, V. ALIANE, D. MANAHAN-VAUGHAN;
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Abstract: Changes in neurotransmission, mediated by the glutamatergic, GABAergic, and dopaminergic systems may underlie pathophysiological changes that lead to psychosis. The hippocampus is severely affected, and alterations in its neurochemistry, structure and function occur. Little is known about the underlying mechanisms. Disruptions of glutamatergic signalling may underlie the pathophysiology of psychosis. We implemented an animal model of psychosis that comprises an acute and single administration of the irreversible N-methyl-D-aspartate receptor (NMDAR) antagonist, MK801 to adult rats, to emulate the onset of first-episode psychosis (Wieschollеck and Manahan-Vaughan, Neuropharmacol, 2013, 74:48). We explored possible changes in glutamate receptor expression, as well as in hippocampal neuronal excitability and synaptic plasticity. Four weeks after MK801-treatment, receptor protein levels were examined using immunohistochemical methods. Cellular compartment analysis by fluorescence *in situ* hybridization was used to measure neuronal activation in learning-naïve animals, or following spatial learning. LTP was assessed in freely behaving rats. In MK801-treated rats, a downregulation of the NMDAR GluN1 subunit occured in the dentate gyrus (DG), whereas an upregulation of mGlu5 receptor in the hilar region, and of mGlu1 receptors in the CA1 cell layer, was evident. These changes were accompanied by a decrease in mGlu2/3 receptors in the Stratum lacunosum moleculare of the CA1 region. No changes were evident for the NMDAR GluN2A and GluN2B subunits. Concurrently, hippocampal LTP was profoundly impaired and animals exhibited significantly higher basal Arc expression in the DG, CA3 and CA1 regions compared to vehicle-injected controls. Interestingly, although spatial learning in controls triggered specific neuronal elevations of Arc, this effect was absent in MK801-treated rats. These findings indicate that in psychosis, deficits in hippocampus-dependent memory may arise through enhanced hippocampal neuronal excitability that are associated with LTP-deficits and altered glutamate receptor expression. These alterations can be expected to result in the disruption of normal information processing that contribute to the cognitive deficits associated with psychosis.

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Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: German Research foundation (DFG)

Title: The 5-Hydroxytryptamine4 (5-HT4) receptor supports differentiated encoding of informational content in the hippocampus

Authors: *H. TWARKOWSKI[1,2], H. HAGENA[1], D. MANAHAN-VAUGHAN[1,2];
[1]Ruhr Univ. Bochum, Med. Fac., Bochum, Germany; [2]Intl. Grad. Sch. of Neurosci., Bochum, Germany

Abstract: Serotonin (5-hydroxytryptamine, 5-HT) contributes to a multitude of physiological processes. At the level of cognition, the 5-HT_4-receptor is particularly relevant: it supports learning and modulates hippocampal synaptic plasticity, in the form of long-term potentiation (LTP) and long-term depression (LTD) at least at the level of the CA1 region (Kemp and Manahan-Vaughan, PNAS, 2014, 101:8192; 2005, Cereb Cortex. 15:1037-43). Interestingly, afferent frequencies that corresponds to $theta_m$ (Bienenstock et al, J Neurosci 1982, 2:32) result in CA1-LTP in the presence of a 5-HT_4-receptor agonist and CA1-LTD in the presence of a 5-HT_4-receptor antagonist (Kemp and Manahan-Vaughan, Cereb Cortex 2005, 15:1037-43). The 5-HT_4-receptor is widely expressed throughout the hippocampus. Here, we explored its contribution to synaptic plasticity at the perforant path-dentate gyrus (PP-DG) and the mossy fiber-CA3 (MF-CA3) synapse. In contrast to the CA1 region, we observed that the $5HT_4$-receptor does not regulate the direction of change in synaptic weights at PP-DG or MF-CA3 synapses, when receptor modulation occurs during stimulation at θm frequencies. However, $5HT_4$-receptor activation *prevents* LTD in both synapses. By contrast, early and late are impaired by $5HT_4$-receptor activation, whereas PP-DG synapses only late LTP is affected We propose that the $5HT_4$ receptor may serve to fine-tune information encoding within the hippocampal circuitry. Thus, information that is encoded by LTP in the DG and particularly in the CA1 region seems to be prioritised. By this means 5-HT_4-receptors may support the differentiation of experience-dependent encoding. This work was supported by a German Research Foundation (DFG) grant to DMV (SFB874, B1)

Disclosures: H. Twarkowski: None. **H. Hagena:** None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Topic: F.02. Animal Cognition and Behavior

Support: German Research foundation (DFG)

SFB874/B1

Title: Exploration of positional or directional spatial cues results in functionally distinct immediate early gene activation in different hippocampal subregions

Authors: *T. HOANG[1,2], V. ALIANE[1], D. MANAHAN-VAUGHAN[1,2];
[1]Dept. of Neurophysiol., Ruhr Univ. Bochum, Med. Fac., Bochum, Germany; [2]Intl. Grad. Sch. of Neuroscience, Ruhr-University Bochum, Bochum, Germany

Abstract: The hippocampus is a key processing and encoding structure for declarative or explicit forms of learning and memory. Hippocampal synaptic plasticity is tightly associated with the encoding and consolidation of spatial and context-dependent memories. Whereas long-term potentiation (LTP) is associated with the acquisition of knowledge about changes in space or global aspects of space in rodents, long-term depression (LTD) is tightly associated with learning about spatial content (Manahan-Vaughan & Braunewell PNAS 1999, 96:8739; Kemp & Manahan-Vaughan, PNAS, 2004, 101:8192; Kemp & Manahan-Vaughan,Trends Neurosci. 2007, 30:111). A functional segregation as to the involvement of sufield-specific LTD in content encoding is also evident: LTD in the dentate gyrus (DG) is enable by landmark content encoding, whereas LTD in the CA1 region is enabled by the encoding of discrete ,or localized aspects of spatial content (Kemp & Manahan-Vaughan, Cereb Cortex, 2008, 18:968). A differentiation with regard to LTD in the CA3 subfield is also evident (Hagena and Manahan-Vaughan, Cereb Cortex, 2011, Cereb Cortex, 21:2442). We postulated that this kind of information encoding by LTD and LTP relates to the 'what' and 'where' streams, and aligns with the parallel map theory of spatial representations (Jacobs & Schenk, 2003, Psychol Rev. 110:285-315). We conducted cellular compartment analysis of immediate early gene activity by means of fluorescence *in situ* hybridization, to assess neuronal activation after novel learning in rats. We detected increases in Arc (or Homer1a) mRNA in the CA1 region after exploration of discrete spatial features and in the dentate gyrus after exploration of large spatially-distinct landmarks. Subfield analysis of the dentate gyrus revealed distinctions in information processing in the upper and lower blades relative to the informational content. These observations are in line with the parallel map theory of spatial representations and indicate that the hippocampal subfields may contribute to functionally distinct components of a spatial representation.

Disclosures: **T. Hoang:** None. **V. Aliane:** None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Topic: F.02. Animal Cognition and Behavior

Support: DFG grant to DMV (MA1843)

Title: The locus coeruleus controls the direction of change and persistency of hippocampal synaptic plasticity through the activation of both ß-adrenergic receptors and dopamine D1/5 receptors

Authors: ***N. HANSEN**[1,2], N. LEMON[2], D. MANAHAN-VAUGHAN[3];
[1]Univ. of Bonn, Bonn, Germany; [2]Neurophysiol., [3]Ruhr-University Bochum, Bochum, Germany

Abstract: Hippocampal long-term depression (LTD) and long-term potentiation (LTP) are cellular mechanism that supports memory formation. Neuromodulation related to arousal is a key factor that determines the effectivity and persistency of hippocampus-dependent memory storage. One important structure in this regards is the locus coeruleus (LC), the activity of which increases in response to novel experience. LC activation facilitates hippocampal LTD at the level of the CA1 region. This is not mediated by noradrenaline release alone: LC stimulation leads to elevations of both dopamine and noradrenaline levels in the CA1 region of behaving rats. Effects are accompanied by LTD and improved spatial learning, and are prevented by both beta-adrenergic receptor antagonists and dopamine D1 receptor antagonists. LC projections to the hippocampus are most dense at the level of the dentate gyrus (DG). Here, we explored LC effects on DG synaptic plasticity. LC stimulation resulted in robust and persistent LTD at perforant-path DG synapses in adult behaving rats. Effects were impaired by an antagonism of ß-adrenoreceptors and dopamine D1/5 receptors. DG long-term potentiation (LTP) was impaired by LC stimulation. Our findings indicate that hippocampal LTD is tightly regulated by noradrenaline and dopamine release from the LC. LTD is promoted, whereas LTP is suppressed by LC activity. We propose that this relates to the role of LTD in content-specific aspects of spatial encoding. This work was supported by a German Research Foundation (DFG) grant to DMV (MA1843)

Disclosures: **N. Hansen:** None. **N. Lemon:** None. **D. Manahan-Vaughan:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOB-1146334

DARPA REPAIR N66001-10-C-2010

Title: Prefrontal connections of the perirhinal and postrhinal cortices in the rat

Authors: *E. HWANG[1], B. S. WILLIS[2], R. D. BURWELL[3];
[1]CLPS, [2]Biotech., [3]CLPS, Neurosci., Brown Univ., Providence, RI

Abstract: Recent work has addressed the role of hippocampal-prefrontal interactions in episodic memory, but other medial temporal lobe structures are also connected with prefrontal regions. Here we report the results of a detailed analysis of the prefrontal connections with the perirhinal (PER) and postrhinal (POR) cortices in the rat. The retrograde tracers were Fast Blue, Diamidino Yellow, and Fluoro-Gold. The anterograde tracers were biotinylated dextrane amine and the plant lectin, Phaseolus vulgaris leucoagglutinin. Injections were located in all parts of PER areas 35 and 36 and the POR. We assessed connections of the secondary motor cortex (MOs), dorsal and ventral anterior cingulate cortices (ACAd and ACAv), prelimbic cortex (PL), infralimbic cortex (IL), and orbital prefrontal cortex (ORB). For each retrograde case we counted labeled cell bodies in each layer of each prefrontal region in a series of coronal sections that were 300 µm apart. For anterograde cases, each layer of each prefrontal region was scored for density of labeled fibers in a series of coronal sections that were 300 µm apart. Preliminary analysis of retrograde tracers shows that PER area 36 receives moderately strong inputs from MOs, and prelimbic cortices, arising in all layers. The density of labeled cells was slightly higher in the MOs than in PL. ACAd provide moderate input to area 36. Superficial layers of MOs, ACAd, PL, and IL provide moderate inputs to PER area 35. Labeling was slightly heavier in the MOs and slightly weaker in the IL. The POR receives a very strong input from all layers of MOs, arising preferentially from caudal MOs. Strong inputs to the POR arise in all layers of ACAd and the deep layers of PL. Prefrontal projections to the PER and POR showed a rostrocaudal topography such that rostral prefrontal areas tended to project to rostral PER and POR, and caudal prefrontal areas tended to project to caudal PER and POR. For the return projections, we found that area 36 provided the strongest projections to prefrontal regions. Fibers were observed in deep and superficial layers of all prefrontal regions examined, but the heaviest labeling was

observed in IL and PL. Area 35 also provided input to prefrontal regions, preferentially to IL. Like the PER, the POR targeted all prefrontal areas. Labeling was weak in all regions except MOs, in which fiber labeling was heavy.

Disclosures: E. Hwang: None. **B.S. Willis:** None. **R.D. Burwell:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Topic: F.02. Animal Cognition and Behavior

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DARPA REPAIR N66001-10-C-2010

Title: Neuronal correlates in rat posterior parietal cortex and the lateral posterior thalamic nucleus during performance on a visuospatial attention task

Authors: *F.-C. YANG[1], R. D. BURWELL[1,2];
[1]Cog., Lin. & Psychological Sci., [2]Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: The posterior parietal cortex (PPC) has been identified as important for visuospatial attention. In rats, this region receives input from visual cortex and from the lateral posterior nucleus of thalamus (LPO), the functional homolog of the primate pulvinar. The LPO and pulvinar are also implicated in attention. In primates, the pulvinar differentially projects to two subdivisions of the PPC. Recent studies showed functional differentiation of these PPC subdivisions, with one region supporting top-down attention and the other supporting bottom-up attention (Cabeza, 2008). The rodent PPC can also be subdivided into dorsal and caudal subdivisions. Whether these subdivisions exhibit a similar functional differentiation, however, remains unknown. Our previous work showed that neuronal activity in dorsal PPC correlated with multiple phases of a visuospatial attention (VSA) task, including onset of the visual stimuli, decision-making, task-relevant location, and behavioral outcome (Yang, Jacobson, and Burwell, under revision). To further understand the roles of the PPC subdivisions and the LPO in visuospatial attention, we simultaneously recorded neuronal activity in all three structures in male Long-Evans rats during performance on the VSA task. This VSA task was adapted from the five-choice serial reaction time task for a double-sided, bowtie shaped enclosure atop the Floor Projection Maze (Jacobson, et al. 2014). Trials alternated from side to side. For each trial, rats

were required to attend to three locations. In one of these locations, a target stimulus was randomly and briefly illuminated. An approach to the correct target location was followed by a liquid reward. For analysis, a trial was divided into behavioral epochs including stimulus onset, selection behavior, and reward. The task was designed to involve both goal-directed, top-down attention and stimulus-driven bottom-up attention. Using wireless recording methods (32-channel TBSI headstage in combination with a Plexon MAP system), we recorded neuronal activity of five rats during performance on the VSA task. We isolated 78 cells in the dorsal PPC, 119 cells in the caudal PPC, and 94 cells in the LPO. Our results show that the LPO and PPC units signal stimulus onset and selection behavior consistent with the interpretation that the LPO and PPC subdivisions are engaged in both top-down and bottom-up visuospatial attention. We also observed LPO and PPC units responded to allocentric and egocentric task-relevant locations. Preliminary analyses showed coherency in the theta frequency band between LPO and PPC subdivisions. Further analyses and detailed results will be presented.

Disclosures: F. Yang: None. **R.D. Burwell:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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NSF IOB-1146334

DARPA REPAIR N66001-10-C-2010

Title: Disconnection of the perirhinal and postrhinal cortices impairs recognition of objects-in-context

Authors: *V. R. HEIMER-MCGINN[1], D. L. POETA[1], R. D. BURWELL[1,2];
[1]Dept. of Cognitive, Linguistics and Psychological Sci., [2]Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: The ability to interpret and use environmental context to inform cognitive processes and guide behavior is an essential function of the human brain. In this study we address the neural basis underlying the formation of object-context associations in the rodent medial temporal lobe. The perirhinal cortex (PER) is known to process object information from the

visual cortex, whereas the rodent postrhinal cortex (POR), homolog to the parahippocampal cortex (PHC) in primates, is thought to process spatial information received from the retrosplenial and posterior parietal cortices. Recent studies provide evidence that representations of context may be formed in the PHC/POR, upstream of the hippocampus. Anatomical data also reveals that the POR and PER are strongly and reciprocally interconnected. Our hypothesis is that the rat POR combines spatial information with object information received from the PER and forms complex representations of context that include the spatial layout of objects and features in the local environment. In this study we employ a crossed lesion approach to test the hypothesis that the POR relies on direct input from the PER in order to provide information about the current context. Rats were tested on two versions of the spontaneous object recognition (SOR) task that measure recognition of objects-in-context (cxtSOR); one is 2-dimensional (2D) and relies exclusively on visual cues, and the other uses 3D objects and relies on multimodal cues. Using the cxtSOR tasks, we examined the effect of crossed excitotoxic lesions to the POR and the contralateral PER. Performance of rats with crossed lesions was compared to that of sham-operated rats and rats with ipsilateral POR plus PER lesions. Our data (n=8 rats/group) show that rats with contralateral POR/PER lesions are impaired in their ability to recognize novel object-context combinations. This is evidenced by a decreased tendency to preferentially explore objects when they are presented in a new context. Discrimination ratios show that rats with contralateral lesions are significantly impaired compared to sham-operated rats in both the 2D and 3D versions of the task. Interestingly, when objects are geometrically simpler in the 2D version of the task, normal rats are less likely to show robust exploration of novelty. These results suggest that interaction between the POR and PER is necessary for the formation of object-context associations, especially when object information is more complex. In further studies, we will use a combination of electrophysiology and optogenetics to specifically inhibit PER input to the POR while studying behavior and neuronal correlates in a cxtSOR task.

Disclosures: V.R. Heimer-Mcginn: None. **D.L. Poeta:** None. **R.D. Burwell:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOB-1146334

DARPA REPAIR N66001-10-C-2010

Title: Contextual dependency of conditioning and extinction in approach and avoidance behaviors

Authors: *T. K. JACOBSON[1], J. R. PHILLIPS[1], R. D. BURWELL[1,2];
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Abstract: Context serves as an important framework for memory, shaping future expectations of experiences in familiar settings, therefore disruptions in the ability to appropriately use contextual information to guide behavior can cause misconstrued meaning of stimuli, inaccurate perceptions, and/or inappropriate behaviors. The context or environment in which learning takes place and the associations formed during learning are fundamentally linked, and yet how and where contextual information processing takes place in the brain is still poorly understood. Several lines of research indicate contextual information processing is deficient in several psychiatric disorders, and many treatments are context dependent. While the traditional fear conditioning paradigms have provided valuable information about the neural bases of both conditioning and extinction of fearful stimuli, they do not address appetitive (reward-seeking) behaviors, behaviors that are repeated for reinforcement (ritualistic behaviors), nor do they address behaviors aimed to avoid anxiety (active avoidance). We have developed a new task that is better suited to investigate the circuits involved in context representations during ritualistic behaviors and also directly compare and contrast the circuitry of context in behaviors that are repeated for reward versus avoidance. The proposed task utilizes the same fundamental principles of conditioning, but rats are trained to associate an approach to a specific visual cue with either reward or to avoid anxiety. We show, as with traditional conditioning paradigms, renewal, reinstatement, and spontaneous recovery of approach behavior, and that it is context dependent. With this new task, we now have a more translatable, clinically relevant model is needed to investigate the underlying circuitry of context.

Disclosures: T.K. Jacobson: None. **J.R. Phillips:** None. **R.D. Burwell:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 724.17/BB1

Topic: F.02. Animal Cognition and Behavior

Title: Impact of cues versus reward location on CA1 place cells

Authors: *F. SHARIF, S. ROYER;
KIST, Seoul, Korea, Republic of

Abstract: Place cells in CA1 are affected by both environmental cues and distance from/to reward locations. How these information reach CA1 cells and integrate is still unclear. We performed silicon probe recording of CA1 cells, in head-fixed mice running on a treadmill belt enriched with visual-tactile cues. Distinct types of cues were used, and each type of cue was repeated in two locations on the belt. A sweet-water reward was delivered on each trial (complete belt cycle) at a given position of the belt, via a lick port. The position of the reward was maintained for 50 consecutive trials, and then changed to a new position, randomly chosen, for the next 50 trials. Mice ran between 150 to 250 trials within a session, hence experienced at least 3 relocations of the reward. We showed previously that, a population of CA1 place cells is tightly controlled by specific cues (cue-cells), as they repeat identical place fields at the duplicated positions of a particular cue (Geiller et al. 2014). We also showed that a population of CA1 place cells 'follow' the reward location, with their firing field maintaining the same distance relation to the reward, while other cells maintain stable firing fields relative to the cues (Koenig et al. 2013). However, in these previous experiments, cue repetition and reward relocation were not tested together, leaving unclear if cells with single fields in the first experiment are the reward-following cells of the second experiment. As expected, a number of cells encoded the duplicated positions of the cues, while other cells followed the location of the reward. Following reward relocation, cue-cells were never converted into reward-following cells or cue-following single place cells. Interestingly, some cells with single place fields were not affected by the reward relocation. Hence, reward distance is not involved in the mechanism generating the unique place field of these cells, which instead, might result from a particular combination of cue-anchored grid cells inputs, or interference between consecutive cues through local interneurons.

Disclosures: F. Sharif: None. **S. Royer:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Topic: F.02. Animal Cognition and Behavior

Support: Intramural Research Program of the NIA

Intramural Research Program of the NIDA

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NIH Grant MH62448

Title: The default mode network in rhesus monkeys with selective bilateral lesions of the hippocampus

Authors: *A. M. SPIEGEL[1], H. GU[3], Y. YANG[3], H. LU[3], J. YOUNG[1], C. HEROLD[1], D. CHARLES[2], S. EDMONDS[2], J. LIVERMORE[2], E. A. MURRAY[4], E. A. STEIN[3], P. R. RAPP[1]; [1]Lab. of Behavioral Neurosci., [2]Comparative Med. Section, Natl. Inst. on Aging, Baltimore, MD; [3]Neuroimaging Res. Br., Natl. Inst. on Drug Abuse, Baltimore, MD; [4]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Human functional imaging studies have identified a distributed network of cortical brain regions that display temporally coordinated activity during wakeful rest, commonly termed the default mode network (DMN). The DMN is largely conserved across rats and nonhuman primates and includes a number of subsystems that converge onto primary DMN core hubs in medial prefrontal and posterior cingulate cortices. The interactions between these hubs and subsystems, and their contribution to the overall integrity and functions of the DMN, remain relatively unexplored. The default mode network and the hippocampus are vulnerable to aging and a variety of age-related neurodegenerative conditions, and here we aimed to directly test the effects of experimental damage to the hippocampus on DMN dynamics. Healthy adult male rhesus monkeys received stereotaxic MRI-guided, bilateral injections of the excitotoxin N-methyl-D-aspartic acid into the hippocampus at 5-6 years of age, approximately 10 years before fMRI. Post-operative structural imaging confirmed the extent and selectivity of the lesions. Sex and age-matched intact adults served as controls. For DMN imaging, monkeys were sedated initially with a single simultaneous cocktail injection of ketamine (3 mg/kg) and dexmedetomidine (0.025mg/kg, IM) and were maintained on a low level of isoflurane (0.5 to 1.0%) during scanning on a Siemens Tim Trio 3T scanner using an EPI sequence (scan parameters: TR =1700ms, TE = 27ms, FOV = 112×112mm, matrix = 64×64, slice thickness = 1.8mm). Initial results confirmed that intact normal monkeys display a temporally coordinated pattern of activity distributed across a number of cortical areas, including the medial prefrontal and posterior cingulate cortex, similar to the human DMN. Ongoing analysis will directly test, for the first time in a primate model, whether the integrity of the hippocampus is necessary for normal resting state cortical network connectivity.

Disclosures: **A.M. Spiegel:** None. **H. Gu:** None. **Y. Yang:** None. **H. Lu:** None. **J. Young:** None. **C. Herold:** None. **D. Charles:** None. **S. Edmonds:** None. **J. Livermore:** None. **E.A. Murray:** None. **E.A. Stein:** None. **P.R. Rapp:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Program#/Poster#: 724.19/BB3

Topic: F.02. Animal Cognition and Behavior

Support: National Institute of Mental Health MH087755 to SSN

Title: Bio-physical model of perirhinal cortex reveals memory storage mechanisms

Authors: *A. ALTURKI[1], P. SAMARTH[1], F. FENG[1], D. B. HEADLEY[2], D. PARÉ[2], S. S. NAIR[1];
[1]Electrical and Computer Engin., Univ. of Missouri, Columbia, MO; [2]Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ., Newark, NJ

Abstract: The perirhinal cortex supports recognition and associative memory. Prior unit recording studies revealed that recognition memory involves a reduced responsiveness of perirhinal cells to familiar stimuli whereas associative memory formation is linked to increasing perirhinal responses to paired stimuli. Both effects are thought to depend on perirhinal plasticity but it is unclear how the same network could support these two opposite forms of plasticity. However, a recent *in vitro* whole brain study (Unal et al., 2012) showed that when neocortical inputs are repeatedly activated, depression or potentiation could develop, depending on the extent to which the stimulated neocortical activity pattern recruited intrinsic longitudinal perirhinal connections. We recently developed a biophysically realistic model of perirhinal area 36 that could reproduce these phenomena. We used the model to shed light on the mechanisms that support associative memory in the perirhinal network. These analyses revealed that perirhinal associative plasticity is critically dependent on a specific subset of neurons, termed conjunctive cells. When the model network was trained with spatially distributed but coincident neocortical inputs, these conjunctive cells acquired excitatory responses to the paired neocortical inputs and conveyed them to widely distributed perirhinal sites via longitudinal projections. Ablation of conjunctive cells during recall abolished expression of the associative memory. However, ablation of conjunctive cells during training did not prevent associative memory formation because a new set of conjunctive cells emerged, revealing that competitive synaptic interactions within the perirhinal network governs the formation of conjunctive cell assemblies. We are presently extending the model to simulate *in vivo* conditions while in parallel incorporating oscillatory potentials into single cell models. Results from these extensions and from model parametric studies will also be presented.

Disclosures: **A. Alturki:** None. **P. Samarth:** None. **F. Feng:** None. **D.B. Headley:** None. **D. Paré:** None. **S.S. Nair:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Program#/Poster#: 724.20/BB4

Topic: F.02. Animal Cognition and Behavior

Support: DHHS/NIH/NIMH/IRP

Title: Automatic expression of recognition memory signals in the perirhinal cortex of rhesus monkeys

Authors: *S. GUDERIAN, M. ROSSA, R. SAUNDERS, M. MISHKIN;
Lab. of Neuropsychology, NIH/NIMH, Bethesda, MD

Abstract: The perirhinal (PRh) cortex is considered to be a critical structure for normal recognition memory function, and neural signals in PRh cortex have been shown to discriminate between new and familiar stimuli in a number of recognition memory tasks. However, the extent to which those signals depend on mnemonic task demands is not well understood. In the present experiment, we asked whether recognition memory signals in the PRh cortex of rhesus monkeys are expressed regardless of whether the old/new status of an image is task relevant, or, alternatively, whether being engaged in a memory task modulates how old and new stimuli are represented in PRh cortex. We trained two adult rhesus monkeys on two tasks each: a recognition memory task and a categorization task. In both tasks, monkeys were presented with a list of dogs and cats, some of which were repeated exactly once. During the recognition memory task, monkeys had to pull a right lever when an image was new, and a left lever when an image was old, regardless of whether an image was of a dog or a cat. During the categorization task, monkeys had to pull a right lever if the image was of a dog, and a left lever if the image was of a cat, regardless of whether it was new or old. This design thus allowed us to assess whether old/new signals are differentially expressed as a function of mnemonic task demand. While monkeys performed both tasks in each daily session, we recorded neural activity in PRh cortex, as well as, simultaneously, in cortical area anteroventral TE. Chronically implanted recording probes consisted of 24 linearly spaced electrodes to span the cortical depth. Spectral analysis of the single-trial current source densities (CSD) revealed that the amplitude of both low (30-60 Hz) and high (60-200 Hz) gamma oscillations discriminated between new and old stimuli in both monkeys, regardless of whether the monkeys were engaged in the recognition memory task or in the categorization task. Specifically, gamma amplitude was higher for new than for old stimuli primarily in the middle layers of PRh cortex, starting approximately 200 ms after stimulus onset. Notably, a similar difference was not observed in area TE. Thus, the present data suggest that the

PRh cortex discriminates between old and new stimuli, regardless of whether the old/new status is task relevant or not. The results will be discussed in light of their implications for our understanding of memory failure, the interaction between memory and attention, and the distinction between episodic and semantic memory.

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Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Program#/Poster#: 724.21/BB5

Topic: F.02. Animal Cognition and Behavior

Support: DHHS/NIH/NIMH/IRP

Title: Impact of Kv7 KCNQ channel blockade in perirhinal cortex on visual recognition memory formation in macaques

Authors: B. CORGIAT[1], D. K. YU[2], A.-J. PISCOPELLO[1], M. MISHKIN[1], *J. N. TURCHI[1]; [1]LN, [2]NIF, NIMH, NIH, Bethesda, MD

Abstract: Direct administration of the muscarinic m1 selective antagonist pirenzepine into perirhinal cortex (PRh) has been shown to produce significant deficits in visual recognition memory in both rats and monkeys. To examine further the muscarinic modulation of visual mnemonic function in PRh, we manipulated an m1-associated K+ channel whose actions could potentially enhance local network connections to support visual memory formation. We conducted local microinfusions of the selective Kv7 KCNQ channel blocker XE 991 in monkeys performing a one-trial visual recognition task and compared these scores to ones following local microinfusions of equivalent volumes of saline or pirenzepine. In sound attenuating operant chambers equipped with touch screen monitors, the animals (n = 3) were first trained to criterion on the delayed nonmatching-to-sample (DNMS) rule using trial-unique clipart stimuli using a 10sec delay between sample and choice test. Next, animals were introduced to varying ratios of clipart to superimposed ascii character stimuli, and both the interstimulus intervals (ISIs) and the stimulus list-lengths were gradually increased. The final task consisted of a list of 16 trial-unique stimuli (clipart:superimposed ascii character stimuli ratio at 25:75) presented at a 15sec ISI, followed by choice tests presented at the same intervals. Thus the final task length required memory of stimuli in the list for a period of 4 - 6 minutes, and each testing session comprised five such lists presented consecutively. On attaining the performance criterion (75-80%

accuracy, no omissions), the animals received a series of bilateral microinfusions into PRh (3.5 µl/site). Relative to saline control infusions, injections of pirenzepine in this region induced visual mnemonic deficits. In contrast, preliminary data from local PRh infusions of XE 991 indicate a dose-dependent positive modulation of recognition accuracy. Our findings provide support for the crucial role of muscarinic m1 receptor-associated pathways in perirhinal cortex for the successful formation of new visual object memories.

Disclosures: **B. Corgiat:** None. **D.K. Yu:** None. **A. Piscopello:** None. **M. Mishkin:** None. **J.N. Turchi:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Topic: F.02. Animal Cognition and Behavior

Support: Grants-in-Aid for Scientific Research on Innovative Areas 26119502

JSPS KAKENHI 15K18358

Title: Cortical and subcortical projections of the entorhinal layer III neurons of the rat

Authors: ***S. OHARA**[1], K. ITO[1], Y. SOTA[1], K.-I. TSUTSUI[1], M. P. WITTER[2], T. IIJIMA[1]; [1]Tohoku Univ. Grad Sch. Life Sci., Sendai, Japan; [2]Kavli Inst. for Sys Neurosci and Cen for Neural Comp, NTNU, Trondheim, Norway

Abstract: The entorhinal cortex is a major input and output structure of the hippocampal formation, and plays an important role in memory and spatial navigation. Various inputs converge in the superficial layers of the entorhinal cortex, and are likely relayed to the hippocampus. Previous anatomical studies reported that the projection to the dentate gyrus and CA3 arises largely from neurons in layer II, while the projection to the CA1 and subiculum originates from neurons in layer III. We have previously examined the multisynaptic inputs to the dorsal and ventral hippocampus of the rat by using rabies virus vector. In line with previous studies, layer II and deep layer III neurons were retrogradely labeled throughout the lateral and medial entorhinal cortex after the viral injection into the dentate gyrus and CA1. However, labeled neurons were hardly observed in the superficial layer III of the entorhinal cortex. In this study, we re-examined the laminar organization of the entorhinal projection neurons in order to identify the efferent projections of the entorhinal neurons located in the superficial layer III. We

injected a retrograde tracer, either a chemical tracer (FluoroGold) or a viral tracer (rabies virus vector), into cortical (medial prefrontal cortex, insular cortex, orbitofrontal cortex, contralateral entorhinal cortex) or subcortical regions (amygdala, nucleus accumbens), and examined the distribution of retrogradely labeled neurons throughout the lateral and medal entorhinal cortex. In line with previous reports, entorhinal-cortical and subcortical projections mainly arose from the deep part of layer III, as well as from layer V. We also confirmed previously published data that superficial layer III neurons project to the contralateral entorhinal cortex. In addition, we found that a small number of superficial layer III neurons projected to the amygdala and the orbitofrontal cortex. These anatomical data indicate that neurons in the superficial half of layer III are connectionally different from those in the deep half.

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Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Title: Characteristic neocortical ensembles encode essential information for different visual shape discriminations

Authors: *A. I. GELLER[1], G. ZHANG[1], H. ZHAO[1], N. COOK[1], M. JAN[1], E. CHOI[1], M. SVESTKA[1], 1. G. COOK[2];
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Abstract: Neural network and synaptic plasticity theories hypothesize that essential information for specific cognitive discriminations is encoded in different neuronal ensembles, in distributed

neocortical networks. However, the critical ensembles are uncharacterized: The size, neuronal composition, and spatial distribution of these ensembles is unknown. Further, we do not know if a specific discrimination is encoded in a characteristic, or different, ensembles across multiple individuals. Here, we used a genetically-modified circuit that encodes essential information for a cognitive task to show that characteristic ensembles encode specific visual shape discriminations. For the model system, protein kinase C (PKC) pathways were activated in several hundred glutamatergic or GABAergic neurons in a critical multimodal associative area, postrhinal (POR) cortex; a constitutively active PKC was delivered using a HSV-1 vector (J Neurosci 2005 25 8468-81). This intervention activated specific PKC substrates with central roles in synaptic plasticity, including glutamatergic neurotransmission or neurotransmitter release, and increased activation-dependent neurotransmitter release. Importantly, this intervention supported enhanced accuracy for specific visual shape discriminations that were learned after gene transfer. Some of the essential information for performance is encoded in the genetically-modified circuit (PNAS 2010 107 14478-83). Following both the gene transfer and learning new image sets, neurochemical lesions that ablated ~21 % of POR cortex, centered on the gene transfer site, selectively reduced performance for only discriminations learned after gene transfer. During learning, there was increased activity for neurons in the genetically-modified circuit, as shown using activity-dependent gene imaging (c-fos and arc). Quantifying the active neurons established that the essential circuit was relatively small, ~500 neurons, and was sparse coded; the coding density of ~3 %, consistent with neural network theories. Analysis of the locations of the active neurons established that different image sets are encoded in characteristic and different ensembles of neurons. Specifically, there was a bilaminar pattern of active neurons: For one image set ([] vs. +) the superficial layer contained more active neurons than the deeper layer; and, in contrast, for a second image set (/ vs. \), the two layers contained similar numbers of active neurons. In conclusion, within a circuit that contains essential information for performance, different discriminations are encoded in characteristic and different ensembles of neurons.

Disclosures: **A.I. Geller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AIG has equity in Alkermes. **G. Zhang:** None. **H. Zhao:** None. **N. Cook:** None. **M. Jan:** None. **E. Choi:** None. **M. Svestka:** None. **1.G. Cook:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 724.24/BB8

Topic: F.02. Animal Cognition and Behavior

Support: RFBR Grant 130440334H

Title: Long-term representation of complex stimuli in the mouse parietal cortex: *in vivo* two-photon c-fos imaging

Authors: *M. ROSHCHINA[1], O. IVASHKINA[1], K. TOROPOVA[1], A. DRONOVA[1], K. ANOKHIN[1,2];
[1]NRC Kurchatov Institute, Nbics-Center, Moscow, Russian Federation; [2]Anokhin Inst. of Normal Physiol. RAMS, Moscow, Russian Federation

Abstract: A common way to study memory of neuronal representations in the rodent neocortex is Pavlovian conditioning to a discrete sensory stimulus. However, in natural environment associative memories are complex and involve integrated conditioned stimuli (CS) consisting of different sensory modalities. Very little is known about how neuronal populations in neocortex code complex stimuli during long-term memory formation and retrieval. Previously we have developed a behavioral paradigm for multisensory compound CS fear conditioning in mice that mimics complexity of natural learning. In the present study, we examined encoding of different components of the compound CS by neurons of the mouse parietal cortex. To map neuronal responses we used transgenic mice with the expression of the EGFP controlled by the c-fos promoter. We trained mice in a fear-conditioning task to a compound cue that consisted of auditory (tone) and visual (blinking light) components. Control mice were presented with the compound CS, without the footshock. A week later, we performed three sequential retrieval sessions during which mice received separate auditory and visual components of the compound CS and the compound CS itself. To monitor retrieval-induced neuronal activation we performed two-photon *in vivo* imaging of fos-EGFP expression in the parietal associative cortex 90 minutes after each test. Based on the intensity of EGFP fluorescence, all registered neurons were divided into the two groups - high- and low-expressing. The number of high-expressing neurons increased in the trained mice compared to the control mice in all retrieval sessions, while the number of low-expressing neurons decreased in all the sessions. Next, we analyzed neurons that showed high fos-EGFP expression only in one of the retrieval session. We found three activation specificities of such neurons: light-related, sound-related and compound CS-related neurons. The number of light-dependent high-expressing neurons increased in the trained mice compared to the control mice. The number of sound-dependent and compound CS-dependent neurons was equal in the trained and control mice. Taken together our data suggests that coding of complex associative memory in the parietal cortex involves at least three neuronal assemblies with different response specificity to the components of the compound CS. This work was supported by RFBR Grant 130440334H.

Disclosures: **M. Roshchina:** None. **O. Ivashkina:** None. **K. Toropova:** None. **A. Dronova:** None. **K. Anokhin:** None.

Poster

724. Learning and Memory: Hippocampus, Rhinal, and Parietal Cortex

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Northwestern University Nicholson Fellowship

Title: Sequential processing of paired stimuli in medial temporal lobe subregions perirhinal cortex, lateral entorhinal cortex, dentate gyrus and CA1 supports formation of a time-bridging associative response

Authors: *E. E. SUTER, C. WEISS, J. F. DISTERHOFT;
Northwestern Univ., Chicago, IL

Abstract: Acquisition of trace eyeblink conditioning (EBC) engages the hippocampus, however the single-neuron responses of perirhinal cortex (PR), lateral entorhinal cortex (latEC) and dentate gyrus (DG) have not previously been described during this task. Rabbits were trained on trace EBC (or given unpaired stimuli; Pseudo) and single-neuron activity was recorded in PR, latEC, DG and CA1 during learning and following a one-month consolidation period. A total of 2891 well-isolated neurons were recorded in PR (N=433), latEC (N=431), dorsal DG (N=1309) and dorsal CA1 (N=718). PR neurons responded to both CS (whisker vibration) and US (corneal airpuff), but showed little conditioning-specific activity. There was however an increase in significantly modulated PR neurons in conditioned animals, consistent with PR as a multimodal coincidence detector that may increase local LTP and transmission to latEC. LatEC and DG showed conditioning-specific trace-period responses starting before behavioral criterion. The magnitude of the stimulus response was reversed between EC responding maximally to CS, and DG firing maximally to US. LatEC showed trace conditioning-induced firing in high-firing rate cells that have not been described during spatial tasks, possibly reflecting local mGluR activation and learning-induced AHP decrease. Thus pre-criterion trace-bridging activity develops in latEC and DG with distinct stimulus-response profiles during temporal learning. PR, latEC and DG neurons showed similar response profiles on CR and no-CR trials, indicating that they are involved in learning associations but not in controlling the behavioral responses--as opposed to

regions such as medial prefrontal cortex, whose neuronal activity differs between CR and no-CR trials. However, the trace-period response in rate-decreasing cells of CA1 is stronger on CR- than no-CR trials. While DG maintained an elevated number of responsive neurons post-consolidation, the characteristic firing rate profiles of EC and DG were diminished post-consolidation, consistent with an acquisition-specific role for these regions. The present study found that within medial temporal lobe, neuronal signals representing paired stimuli are transformed in a step-wise process from individual stimuli in PR, to stimuli plus trace-bridging activity in EC and DG, to a behavior-linked response in CA1.

Disclosures: **E.E. Suter:** None. **C. Weiss:** None. **J.F. Disterhoft:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 725.01/BB10

Topic: F.02. Animal Cognition and Behavior

Support: RIKEN Brain Science Institute

Kakenhi

Title: CA1 rate and temporal coding in the absence of CA3 input

Authors: S. J. MIDDLETON[1], *T. J. MCHUGH[2,3];
[1]Riken Brain Sci. Inst., Wako-Shi, Saitama, Japan; [2]RIKEN Brain Sci. Inst. - Wako, Wako-Shi, Saitama, Japan; [3]Dept. of Life Sciences, Grad. Sch. of Arts and Sci., Univ. of Tokyo, Tokyo, Japan

Abstract: Area CA1 of the hippocampus is known to receive temporally patterned inputs from area CA3 (via the Schaffer collaterals) and layer 3 of the entorhinal cortex (via the temporoammonic pathway). These inputs give rise to spatial coding in CA1 which manifests as both rate: a spatially selective increase in firing rate as the animal enters and traverses the cells receptive field and temporal coding: precise timing of spike discharge relative to hippocampal local field potential theta oscillations. Whilst the anatomical inputs to CA1 are well characterized, their respective contributions to rate and temporal coding are less clear. Using the CA3-TeTX transgenic mouse, in which CA3 output can be specifically and inducibly silenced we dissected to what degree the two types of coding are utilized by CA1 devoid of CA3 input.

Our results show that phase precession, the discharge of spikes at progressively earlier theta phases with place field distance, remains intact despite loss of CA3 input.

Disclosures: S.J. Middleton: None. **T.J. McHugh:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

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Support: ANR-13-JSV4-0002-01

Ville de Paris - Emergences 2013

Title: An examination of the local circuitry and impact on network activity by supramammillary nucleus inputs to area CA2 of the hippocampus

Authors: *R. A. PISKOROWSKI, V. ROBERT, V. CHEVALEYRE, L. THERREAU; CNRS UMR8118, Paris, France

Abstract: Area CA2 of the hippocampus differs from areas CA1 and CA3 in many aspects. Recent findings indicate that this region is unlikely to code spatial information, but is critical for social memory. Neurons in area CA2 form a reciprocal connection with the supramammillary nucleus (SuM), a hypothalamic structure activated by stress and reward. We are using targeted viral vectors in combination with transgenic mouse lines in order to selectively express channelrhodopsin in SuM neurons and selectively excite projections from these neurons in transverse hippocampal slices. We have found that SuM fibers form excitatory synapses onto both pyramidal cells and interneurons in the deep portion of the somatic layer in area CA2. An inhibitory post-synaptic potential is evoked in CA2 pyramidal cells following SuM stimulation, which is entirely abolished after blocking excitatory transmission. These results reveal that SuM fiber stimulation effectively evokes action potentials in interneurons that feed-forward onto CA2 pyramidal cells. In contrast, the direct glutamatergic transmission between SuM and CA2 pyramidal cells is quite weak and unable to evoke firing. Furthermore, SuM activity results in the release of neuropeptide, allowing for an indirect modulation of inhibitory transmission in this area. We are examining the properties and axonal projections of the interneurons that receive inputs from SuM fibers in order to better understand how SuM activity alters the local circuitry

in the hippocampus. We postulate that strong recruitment and modulation of perisomatic inhibition in this area may influence network oscillatory activity.

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Poster

725. Learning and Memory: Hippocampal Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: RIKEN Brain Science Institute

Title: The role of CA2 in regulating information flow in the hippocampus

Authors: *R. BOEHRINGER[1], D. POLYGALOV[1], A. J. Y. HUANG[1], R. A. PISKOROWSKI[2], V. CHEVALEYRE[2], T. J. MCHUGH[1,3];
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Abstract: Understanding the flow and processing of spatial and contextual information across the subregions (CA1/CA2/CA3/DG) of the hippocampus has been a long standing goal of neuroscience and has provided key insights to the formation, consolidation and expression of declarative memory. Key to this understanding has been the combination of *in vivo* recording of the spatially selective place cells and local field potential oscillations across the structure with modern genetic tools that allow interventions in neuronal function at specific nodes of the circuit. While this approach has yielded insights into the contributions of CA1, CA3 and the DG, the role of the small, yet highly connected CA2 region to spatial processing and circuit function remain largely unexplored. CA2 is unique in its pattern of synaptic plasticity and its anatomy, possessing multiple bidirectional connections with areas both within and outside the hippocampus, including layer II of the entorhinal cortex, CA3, the supramammillary nucleus of the hypothalamus, and the medial septum. Using *in vivo* recordings in awake behaving mice we have found that while many aspects of CA2 physiology are similar to the neighboring CA3 region, the consequences of CA2 silencing are quite unique. Using multiple genetically encoded systems we have investigated the consequences of both transient and chronic silencing of CA2 synaptic

transmission on hippocampal physiology. Surprisingly, our data suggests that CA2 may serve a crucial role as a regulator of interhippocampal information flow.

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Poster

725. Learning and Memory: Hippocampal Circuits

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Kakenhi Grant 15K14357

Title: Supramammillary input to hippocampal CA2 modulates social memory

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Abstract: The individual pyramidal cell subfields of the hippocampus (CA1/CA2/CA3) can be differentiated by their anatomy, physiology and contributions to specific types of memory processing. Recent work has demonstrated that chronic genetic silencing of synaptic transmission specifically in CA2 pyramidal cells resulted in a loss of social memory. The CA2 region is unique in that it is the only of the CA regions to receive direct projections from the hypothalamus; both the supramammillary (SuM) nucleus and the paraventricular nucleus send axons that synapse specifically in CA2. As a large body of previous work has highlighted the importance of the hypothalamus in social and affective behaviors we have begun to address the role of the hypothalamic-hippocampal circuit in modulating CA2-mediated social memory. We have generated a new Cre recombinase expressing transgenic mouse line that permits specific genetic access to projection neurons of the SuM. Stereotaxic injection of a cre-dependent AAV virus expressing optogenetic channels into the SuM of this cre line, combined with laser stimulation of terminals in CA2, provides us with a novel tool to address the role of these inputs

in CA2 physiology and memory, both *in vivo* and *in vitro*. Physiological data indicate that SuM inputs generate a complex circuit response, with both rapid and slow components. Moreover, terminal stimulation of the SuM projections in CA2 during behavior inhibits the expression of social memory, suggesting that the SuM may serve as a signal of social novelty. These experiments shed light on a novel hypothalamic-hippocampal circuit that regulates the memory of social interaction and have begun to uncover why CA2 may be a key player in this network.

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Poster

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RIKEN Brain Science Institute Junior Research Associate

KAKENHI 25116529

Title: Hippocampal CA3 plasticity and transmission are required for social memory

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Abstract: Social recognition and memory are crucial for normal behavior in many animals, including rodents and primates, however, the neural circuits underlying these processes are not fully understood. Recent results have suggested an important role of the hippocampus in social memory, with a particular focus on the CA2 subregion. Here we addressed the potential contribution of the hippocampal regions upstream of CA2, CA3 and the dentate gyrus (DG). We found the deletion of the NMDA receptor subunit 1 gene (NR1), which abolishes NMDA receptor synaptic plasticity, specifically in CA3 pyramidal cells led to deficits in social memory, however, mice lacking the same gene in DG granule cells performed indistinguishably from controls. In addition, we have used transgenic and viral techniques to demonstrate CA3 synaptic

output is required for normal social memory. Together, our results provide new evidence that disruption of hippocampal CA3 function contributes to deficit in social memory in mice, and suggests an interaction between the CA3 and CA2 circuits may be required for the acquisition and expression of social memory.

Disclosures: **M. Chiang:** None. **A. Huang:** None. **T.J. McHugh:** None.

Poster

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NIH Grant R01 MH094146

JHU Brain Sciences Institute Grant

Title: CA2 place cells maintain a coherent population representation of a dynamically changing environment

Authors: *H. LEE, C. WANG, S. DESHMUKH, J. J. KNIERIM;
Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: The hippocampal CA2 area was historically considered a transition zone between CA3 and CA1. However, growing evidence suggests that CA2 has unique connectivity and physiology that may contribute important roles in hippocampal processing and computation. To examine the neural representations of a dynamically changing environment within the CA2 area, we recorded CA2 neurons during a cue-shift rotation experiment. Rats were trained to run around a circular track containing salient local cues on the track in a room containing salient global cues on the walls. Three standard sessions were interleaved with two mismatch sessions, in which the local cues on the circular track were rotated counterclockwise (CCW) and the global cues in the room were rotated clockwise (CW) to result in total mismatch angles of 45°, 90°, 135°, or 180°. The individual cellular response to the double rotation manipulation showed that the majority (67%) of CA2 place fields rotated on the track (CCW, n = 80/173; CW, n = 26/173; ambiguous rotation, n = 10/173). The remaining place cells remapped their place fields, having a robust place field in only one of the two sessions (appear and disappear, n = 57/173). A population correlation analysis showed that the CA2 representations of the standard and the

mismatch environments were strongly correlated and that the representations were controlled by the local cues. The coherence in CA2 was similar to prior findings in intermediate and distal CA3 populations recorded in the same double rotation experiment, but different from the adjacent, proximal CA1 representation. Thus, under these conditions, the neural processing in CA2 resembles CA3 more than CA1.

Disclosures: **H. Lee:** None. **C. Wang:** None. **S. Deshmukh:** None. **J.J. Knierim:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: R01 MH094146

R01 NS039456

Title: Functional dissociation along the proximodistal axis of CA1

Authors: ***S. S. DESHMUKH**[1,2], J. L. JOHNSON[2], J. J. KNIERIM[2];
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Abstract: Lateral and medial entorhinal cortex (LEC and MEC) projections are segregated along the proximodistal axis of area CA1 of the hippocampus. LEC preferentially projects to distal CA1, while MEC preferentially projects to proximal CA1. Previous experiments in our lab have shown that in response to a conflict between local (on a circular track) and global (along the circular curtain) cues, the response of the CA1 place-cell population is incoherent: some neurons rotate their place fields with the local cues, others with the global cues, and the majority remap (Lee et al., 2004). We asked if this apparent lack of coherence can be explained by a functional dissociation along the proximodistal axis of CA1 due to functionally different inputs received from LEC and MEC (Deshmukh and Knierim, 2011). We recorded from 11 rats with 16-18 tetrodes spanning the entire transverse axis of dorsal CA1 while the rats were subjected to the double cue-rotation paradigm. In addition, we included data from CA1 neurons from 9 rats reported from in previous studies. The electrodes in each of these rats targeted only a part of the transverse axis of dorsal CA1. The data from these two sets of rats were qualitatively similar and hence pooled. Analysis at the single neuron level as well as at the population correlation level

revealed a difference in cue preference along the proximodistal axis of CA1. The distal CA1 representation was coherently controlled by the global cues. In contrast, the proximal CA1 representation showed a less coherent response, with a stronger influence of local cues. Intermediate CA1 showed varying preference for local and distal cues depending on the mismatch angle. These data demonstrate functional heterogeneity along the transverse axis of CA1 in the responses of place cells to spatial reference frame conflicts.

Disclosures: S.S. Deshmukh: None. **J.L. Johnson:** None. **J.J. Knierim:** None.

Poster

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HFSP Fellowship LT00683/2006-C

Title: Multiscale simultaneous response of grid cells to conflicting reference frames

Authors: *F. SAVELLI, J. D. LUCK, J. J. KNIERIM;
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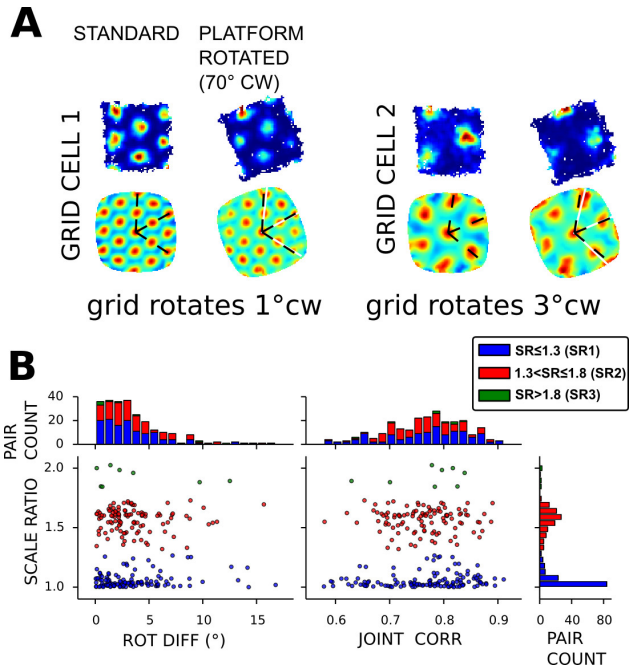
Abstract: Grid cells represent space over multiple, discrete spatial scales. Grids of the same scale share common geometrical features (e.g., orientation and elliptical distortion), which can differ across grids of distinct scale. While grid cells of the same scale are also known to maintain rigid geometric coupling across remapping-inducing experimental manipulations, coupling (or lack thereof) across distinct grid scales remains experimentally under-characterized despite its theoretical importance. We previously described experiments in which a proximal reference frame (foraging platform) and a distal frame (experimental room) were dissociated by translation or rotation in separate foraging sessions. These manipulations elicited diverse geometric adaptations by grid cells, ranging from locking to either frame to the disruption of grid structure. Pairs of co-recorded grid cells of both same and different scale from two rats (208+35 pairs) were analyzed here. For each pair-session, we measured (1) the difference in grid rotation (**A)** and (2) a joint correlation of the pairs (stacks) of grids across the standard and manipulated condition, under a single, rigid affine transformation (cosine of the angle between linearized

stacks, after rate maps are individually mean-centered and normalized). Pairs were divided into 2-3 discrete spacing-ratio clusters (SR1-3; n=126, 109, 8; **B**). 77% (80%) of SR1 (SR2) grid pairs displayed <5° rotation difference and 80% (83%) had joint correlation >0.7 (**B**). Neither rotation difference nor joint correlation was found to be significantly different in SR1 vs. SR2 (Rotation Difference: p>0.5, 2-sided Mann-Whitney test, both medians= 2.8°; Joint Correlation: p>0.24, medians=.78, .76). We did not find unambiguous examples of grid dissociation by visual inspection of rate maps. In these animals and experimental protocols, the responses of co-recorded grids to conflicting reference frames appeared to be generally well coordinated and independent of their scale ratio.



Disclosures: **F. Savelli:** None. **J.D. Luck:** None. **J.J. Knierim:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: R01 MH094146

Title: Landmark vector coding in complete darkness in area CA1 of the hippocampus

Authors: *V. PULIYADI, S. S. DESHMUKH, J. L. JOHNSON, J. J. KNIERIM;
The Johns Hopkins Univ., Baltimore, MD

Abstract: Animals have been shown to utilize the distance and direction from landmarks to find hidden food rewards. After gerbils are trained to use two landmarks to locate buried food rewards equidistant from each landmark, moving the landmarks causes the gerbil to search in spots with the same distance and direction from each landmark (Collett et al. 1986). McNaughton et al. (1995) proposed a vector encoding model to explain this behavior, suggesting that individual place fields can generate a vector with orientation and distance from a specific landmark. Instances of such landmark-vector encoding were identified in previous recordings from our lab in the CA1 hippocampal subregion (Deshmukh and Knierim, 2013). Units with this property are defined as having place fields that maintain a specific and consistent vector relationship to specific landmarks in the environment. Since these fields are object-related but located away from the objects, visual cues would be expected to perform an important role in the generation and maintenance of these landmark vectors. To investigate the necessity of visual information, the neural activity of CA1 place cells was recorded while rats foraged on a circular platform (48" dia.) in darkness, with three novel objects set in an equilateral triangle centered on the platform. The rats had never experienced the platform in the presence of light. This paradigm was repeated for three days without any manipulation of the objects. On the fourth day, a session in which the cues were rotated by 60° was interleaved with the standard object configuration sessions. Of 458 units from seven rats, 7.5% were identified as encoding vector relationships between the rat and a subset of the landmarks, a proportion similar to the prior report in the light (Deshmukh and Knierim, 2013). These results suggest that the hippocampus can encode space relative to objects without the necessity of visual information even when the rat is not in contact with the objects.

Disclosures: V. Puliyadi: None. **S.S. Deshmukh:** None. **J.L. Johnson:** None. **J.J. Knierim:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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Title: Place cells in virtual reality dome reveal interaction between conflicting self-motion and landmark cues

Authors: *M. S. MADHAV[1], R. P. JAYAKUMAR[2], F. SAVELLI[1], H. T. BLAIR[3], N. J. COWAN[2], J. J. KNIERIM[1];
[1]Mind / Brain Inst., [2]Mechanical Engin., Johns Hopkins Univ., Baltimore, MD; [3]Psychology, UCLA, Los Angeles, CA

Abstract: The generation of allocentric 'cognitive maps' in the hippocampal formation requires that positional information derived from external landmarks be reconciled with self-motion information derived from vestibular, proprioceptive, and other cues. Using a custom, hemispherical, planetarium-style virtual reality (VR) dome, we are investigating the interactions between landmarks and self-motion cues on place cells and other neural correlates believed to underpin the cognitive map. Inside the VR dome, a rat can traverse a circular path (4.5 ft. dia.) and unlike most existing rodent VR systems, the rat experiences naturalistic inertial and proprioceptive feedback, while only the visual feedback is artificially controlled. The surrounding visual scene consists of salient virtual landmarks, which are rotated coherently as a function of the rat's angular position, creating the visual illusion that the rat is walking slower or faster, depending on the sign of the landmark angular velocity. The ratio of applied landmark angular velocity to measured rat angular velocity is termed the "gain" (g) of visual feedback; in particular, g=0 corresponds to stationary landmarks and g=1 corresponds to landmarks moving along with the animal. Preliminary data demonstrated the formation of stable CA1 place fields in the presence of three stationary virtual landmarks. These place fields drifted coherently when the landmarks were turned off, mitigating concerns of interference from uncontrolled, non-VR directional cues in our apparatus and indicating that, as expected, the animal's internal representation of position accumulates error when relying solely on self-motion inputs. Continuous manipulation of visual landmarks ($0<|g|<0.9$) reliably controlled the firing location of place cells, even though the landmarks were radically displaced from their initial locations by the end of the experiment. However, at the higher gains ($g>0.5$), initial observations indicated that firing fields progressively increased in size. In addition, the fields shifted backwards relative to the location of the landmarks by up to 100 degrees, possibly due to competition between conflicting place estimates from landmark and self-motion cues. Our ongoing efforts aim at elucidating the mechanistic significance of these preliminary findings as well as further pursuing the unique experimental opportunities afforded by our apparatus to understand the computational and sensory interplay of allothetic vs. idiothetic spatial cues in a highly controlled and real-time fashion.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: RO1 NS039456

Johns Hopkins University Brain Science Institute

Title: Neurons in the hilus of the dentate gyrus have multiple place fields in multiple environments

Authors: D. GOODSMITH[1], *K. M. CHRISTIAN[2,3], S. KIM[2,3], H. SONG[2,3,4], J. J. KNIERIM[1,4];
[1]Krieger Mind/Brain Inst., [2]Dept. of Neurol., [3]Inst. for Cell Engin., [4]The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Early computational models suggested that the dentate gyrus (DG) orthogonalizes overlapping input patterns from entorhinal cortex by recruiting independent ensembles of sparsely firing granule cells (GCs). This notion of "expansion recoding" has been challenged by evidence that many putative GCs have multiple firing fields and fire promiscuously in multiple environments (Leutgeb et al, 2007; Jung & McNaughton 1993). This result suggested that an active population discriminates environments (or input patterns) based on changes in the spatial or temporal coincidence of firing, rather than the sparse activation of discrete subsets of cells. In a more recent study, Neunuebel & Knierim (2012) found that tetrodes in the DG can record at least two cell types with distinct firing properties. One group of cells was rarely active in behavior and had single fields when active, consistent with the formation of a representation of the environment based on different populations of sparsely firing cells. The other group of cells was more likely to fire during behavior, had multiple fields, and was recorded on tetrodes with fewer cells active during sleep. These cells resembled the putative multi-field GCs in previous studies and were often recorded on tetrodes in the hilus; however, it was unclear if these hilar cells would also fire in multiple environments. We recorded from the DG while rats foraged for food in four distinct environments. Four unique platforms (different textures, colors, and/or shapes) were placed in four different rooms with distinct external cues. During long periods of sleep or quiet inactivity (30-120 min) in 3 rats, 13 cells were recorded on 5 tetrodes histologically identified to be located in the hilus on the day of recording. During subsequent foraging behavior, 11 of these cells (85%) had ≥ 1 place fields in all environments, and only one cell was silent in all environments. These cells had an average of 2.55 place fields per active room (range 1-5). The spatial firing of these cells in the hilus resembles the firing of cells

attributed to the granule cell layer in previous studies. Along with Neunuebel & Knierim (2012), the present results show that cells in the dentate gyrus with multiple place fields in multiple environments may be mossy cells or other hilar cell types.

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Poster

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Support: R01 MH094146

R01 NS039456

Title: Deep-layer LEC neurons convey slightly more spatial information than superficial-layer neurons in open-field foraging tasks

Authors: *C. WANG[1,2], G. RAO[1,2], H. LEE[1,2], J. J. KNIERIM[1,2];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Mind/Brain Inst., Baltimore, MD

Abstract: The hippocampus, which plays an essential role in episodic and spatial memory, receives its major cortical input via the superficial layers of the entorhinal cortex. The superficial layers of the medial entorhinal cortex (MEC) contain many spatially selective neurons (e.g., grid cells and boundary cells), whereas the superficial layers of the lateral entorhinal cortex (LEC) do not appear to contain many cells with robust spatial selectivity. The LEC superficial layers receive projections from other high-order sensory and association cortices, whereas the deep layers are targeted by feedback projections from the hippocampus proper, including CA1 place cells. We tested whether deep-layer LEC neurons contain more spatial information than superficial-layer LEC neurons. We trained 7 rats to forage freely in open fields in rooms with rich visual cues and measured the spatial information content of recorded neurons. There were small but significant differences in spatial information score between the firing rate maps of superficial- and deep-layer cells (Superficial: n = 114, median = 0.13, interquartile range = 0.08-0.21; Deep: n = 42, median = 0.20, interquartile range = 0.11-0.28; Wilcoxon rank-sum test, $z = 2.42$, $p < 0.02$). Thus, deep-layer LEC cells, which receive spatially selective input from CA1 backprojections and from subiculum, show slightly more spatially selective firing than

superficial-layer cells. Nonetheless, the spatial selectivity of the deep-layer cells is much lower than prior reports of hippocampal place cells and MEC grid and boundary cells, thus reinforcing the distinct functional differences among these 3 components of the hippocampal memory system. The results suggest that deep-layer LEC recodes the space-based representation it receives from the hippocampus, which may facilitate communication between neocortical regions and the hippocampus proper.

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Poster

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Support: NIH R01AA016852

Title: Optogenetic manipulation of memory encoding in primate hippocampus

Authors: ***I. OPRIS**, L. SANTOS, D. KLORIG, D. FETTERHOFF, D. W. GODWIN, R. E. HAMPSON, S. A. DEADWYLER;
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Abstract: Prior findings from this lab have demonstrated facilitation of cognitive performance in nonhuman primates trained to perform in a delayed match to sample (DMS) task (Hampson et al., 2013). Our objective is to develop a memory prosthesis controlled by light. Therefore, we hypothesized that optogenetic stimulation (OS) in primate hippocampus is capable of modifying (enhancing or decreasing) memory encoding. To test the role of hippocampal microcircuits in memory encoding we combined OS with simultaneous recording of hippocampal subfields CA3 & CA1. OS experiments were carried out in four rhesus macaques, previously injected in CA1 with adeno-associated viral vector AAV5 (Diester et al. 2011), carrying an excitatory opsin, channelrhodopsin-2 (ChR2, blue light) and an inhibitory halorhodopsin (HR, yellow light). OS of hippocampal neurons is performed by applying laser stimuli during the Sample phase of the DMS task when the memory was encoded. Simultaneous neural recording and OS were performed using a custom coaxial tetrode developed in our lab (Santos et al., 2012). We found that neural activation patterns revealed by OS alone were determined in part by the functional connections of this region expressing ChR2 or HR. Thus, stimulation of the CA1 subfield containing ChR2 led to the activation of neighboring hippocampal cells, while stimulation of the

CA1 subfield containing HR led to the inhibition of these cells. When optogenetic stimulation was combined with visual stimulation in the task, the observed neural activation patterns revealed a complex interaction between the two stimuli (laser and task image), inducing facilitation or inhibition that depended on the stimulus type of visual stimulus presented and the timing of visual and optogenetic stimulus presentation. Our results demonstrate (1) the use of optogenetics to stimulate specific functional microcircuits in hippocampal CA1 of a non-human primate; (2) changes in neural activation and/or microcircuit state following optogenetic stimulation that facilitate or inhibit neural cell firing; and (3) differential modulation of firing in hippocampal cells encoding spatial or object features of visual stimuli. These investigations have relevance for understanding the behavioral, neurophysiological, neuropathological and neurochemical abnormalities that occur in patients with dementia, epilepsy or schizophrenia. The outcome of these results has groundbreaking implications for the design of cognitive prostheses controlled by light for patients exhibiting a broad spectrum of cognitive dysfunctions.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01 MHO86591

Title: Inactivation of the C57BL/6J mouse hippocampus disrupts discrimination and avoidance of objects that are either stationary or moving around the environment

Authors: *H. N. ASGEIRSDOTTIR[1], R. W. STACKMAN, Jr.[2];
[1]Psychology, [2]Florida Atlantic Univ., Jupiter, FL

Abstract: The cognitive map theory states that the hippocampus creates representations of locations where relevant non-spatial items or objects are encountered and where specific events occur within a contextual or spatial reference frame. The rodent hippocampus is an essential neural substrate for spatial memory and the CA1 region has been shown to play a vital role in object memory dependent and independent of context; findings consistent with the cognitive map view. Ongoing identification of an object and continuous updating of its location within the environment combines these hippocampal dependent functions. How the hippocampus integrates

these two types of information in real life situations has not been well studied. In particular, it is of interest to determine how the rodent hippocampus processes information about 3D objects-in-motion. Here, we trained mice to avoid an object under both stationary and moving conditions by delivering a mild foot shock when in proximity to the object. Intrahippocampal infusion of muscimol impaired avoidance of the object during both testing conditions; results which support the view that an intact hippocampus is required for both object memory retrieval and continuous updating of the objects' location in space. Next, we developed a novel behavioral paradigm to further test the influence of motion on hippocampal-dependent object discrimination. Here, we trained mice to avoid one object while not avoiding another one, and then tested whether the mice can discriminate between moving the objects during hippocampal inactivation. The "Knowing your enemy" task requires the mice to discriminate between two moving objects and successfully evade proximity to only one of them to avoid receiving a foot shock. This task requires mice distinguish between the objects and continuously update their location in space, both of which are believed to be hippocampal dependent. Temporary inactivation of the hippocampus in mice after training of the "knowing your enemy" task resulted in impaired avoidance and increased exploration of both objects while stationary. These results support involvement of the rodent hippocampus in non-spatial object memory.

Disclosures: H.N. Asgeirsdottir: None. **R.W. Stackman:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 24530909

KAKENHI 26590174

Title: Hippocampal NMDA receptors are involved in rats' spontaneous object recognition only under high memory load condition

Authors: *K. YAMADA, M. SUGITA, Y. ICHITANI;
Univ. Tsukuba, Tsukuba, Japan

Abstract: Involvement of the hippocampus in non-spatial memory, such as recognition memory for items or objects, is still controversial. It has been reported that the hippocampus could be

involved in spontaneous object recognition under a specific condition such as a longer delay length, although some previous studies have argued that the hippocampal lesions had no effect on performance in the spontaneous object recognition test. Thus, the hippocampus seems to be involved in item or object recognition memory only under a higher memory load condition. In this study, the possible involvement of hippocampal N-methyl-D-aspartate (NMDA) receptors in spontaneous object recognition was investigated in rats under different memory load conditions. We firstly estimated rats' object memory span using 3 to 5 objects in "Different Objects Task (DOT)" in order to confirm the highest memory load condition in the objet recognition memory. Long-Evans male rats were allowed to explore a field in which 3 (3-DOT), 4 (4-DOT) or 5 (5-DOT) different objects were presented. After a delay period, they were placed again in the same field in which one of the sample objects was replaced by another object, and their exploration behavior to these objects was analyzed. Rats could discriminate the novel object from the familiar ones in 3-DOT and 4-DOT but not in 5-DOT, suggesting that rats' object memory span was about 4. Then we examined the effects of hippocampal AP5 infusion on performance in both 2-DOT (2 different objects were used) and 4-DOT. The drug treatment before the sample phase impaired performance only in 4-DOT. These results suggest that hippocampal NMDA receptors play a critical role in spontaneous object recognition only when the memory load is higher enough.

Disclosures: **K. Yamada:** None. **M. Sugita:** None. **Y. Ichitani:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 725.16/BB25

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R00 MH083943

NIH Grant R01 2616787650

Title: Bidirectional modulation of context fear in the dentate gyrus

Authors: ***B. E. BERNIER**, H.-J. KIM, A. AYOUB, B. V. ZEMELMAN, M. R. DREW; Ctr. for Learning and Memory, Univ. Texas At Austin, Austin, TX

Abstract: Recent studies have demonstrated the importance of the hippocampal dentate gyrus (DG) in the acquisition of contextual fear memories. However, the neural substrates underlying

extinction of context fear have not been clearly defined. Here, we use optogenetic and pharmacogenetic methods to assess the role of DG in context fear extinction in mice. To rapidly and reversibly manipulate neural activity during behavior, an adeno-associated viral (AAV) vector was used to express the light-activated chloride pump halorhodopsin (eNpHR3.0) specifically within the DG. Optogenetic inhibition of the dorsal DG during the context-shock pairing impaired context fear acquisition. Silencing the DG during context re-exposure did not alter fear expression but attenuated fear extinction across 5 days of context re-exposure. Silencing the DG only during a context re-exposure after extinction failed to increase freezing, suggesting that DG is not required for expression of extinction learning. The data suggest that DG is required for acquisition of new context learning (both acquisition and extinction) but is not required for expression of context learning. To further examine the role of the DG in context learning, we used an AAV vector to express the engineered G protein-coupled receptor rM3Ds in excitatory neurons of the dorsal DG. Activation of rM3Ds via injection of clozapine-N-oxide (CNO) stimulates the cAMP pathway, leading to increased activation of dentate granule cells (DGCs) during behavior. Increasing activity of DGCs during context-shock pairing impaired acquisition of context fear, similarly to DG inhibition. Increasing excitability of DGCs during context re-exposure after acquisition had no effect on expression of the fear memory but led to a significant reduction in freezing during a subsequent drug-free context test. This reduction in freezing does not reflect an enhancement of extinction learning, as stimulating DGC activity with CNO during exploration of a novel context 24 hours after conditioning produced a similar decrement in freezing during subsequent re-exposure to the conditioning context. This finding suggests that stimulating DGCs after conditioning reduces fear by interfering with mechanisms related to consolidation. Together these data identify the dorsal DG as a critical component in the neural circuitry of context extinction and a potential therapeutic target for the treatment of emotional disorders related to aberrant fear learning.

Disclosures: **B.E. Bernier:** None. **H. Kim:** None. **A. Ayoub:** None. **B.V. Zemelman:** None. **M.R. Drew:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

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Program#/Poster#: 725.17/BB26

Topic: F.02. Animal Cognition and Behavior

Support: MH 46904

MH 74006

Title: Dentate Gyrus is required for the acquisition, but not the expression, of trace eyelid conditioning in mice

Authors: *Y. KIM, B. BERNIER, M. DREW, M. MAUK;
The Univ. of Texas At Austin, Austin, TX

Abstract: Decades of studies supported a contribution of the hippocampus for various types of learning and memory. The tri-synaptic circuit, which is thought to be the heart of hippocampal information processing within hippocampus, conveys information from dentate gyrus (DG) to CA3 to CA1. The three subregions are distinct in terms of the composition of cell types, the basal activity of principal neurons, input sources and output targets, suggesting that their contribution to the hippocampal functions may not be the same. In this study, we tested the contribution of DG using trace eyelid conditioning in head-restrained mice. Previous studies using permanent lesion of the whole hippocampus have shown that the hippocampus is necessary for acquisition of trace eyelid conditioning and for the expression of the recently acquired conditioned responses (CRs). To inhibit DG selectively, we expressed halorhodopsin in DG either by injecting AAV containing eNpHR3.0-YFP in naïve C57BL/6J mice or by crossing POMC-Cre mice with floxed- eNpHR3.0-YFP mice. Trace conditioning involved a 300 ms tone CS and periorbital stimulation as the US. The US was presented 500 ms after the offset of the tone CS (i.e. 500 ms trace interval). With this training the animals acquire reliable CRs (>85% of total trials) within seven days. Muscimol infusion in the pons and deep cerebellar nuclei abolished CRs, suggesting that this is cerebellum-dependent eyelid conditioning as reported with rabbits, rats, and mice. Halorhodopsin-expressing mice did not show CRs when DG was inactivated during each trial in the acquisition sessions, indicating that DG activity is necessary for the acquisition of trace eyelid responses. In contrast, these mice can learn comparable to control (GFP-expressing mice or littermates) when laser light is blocked, suggesting that the DG is not simply blocking the expression of CRs. Once animals were conditioned, the inhibition of DG did not affect the expression of CRs. These results suggest that DG is necessary for the acquisition, but not the expression, of trace eyelid responses.

Disclosures: **Y. Kim:** None. **B. Bernier:** None. **M. Drew:** None. **M. Mauk:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

Location: Hall A

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Program#/Poster#: 725.18/BB27

Topic: F.02. Animal Cognition and Behavior

Support: UBC Psychology startup funds

Title: Spatial memory functions for adult neurogenesis are modulated by stress in male and female rats

Authors: T. P. O'LEARY, *J. S. SNYDER;
Dept. of Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The discovery that new neurons are born in the adult brain has opened the door to exciting possibilities by which experience can sculpt circuits and modify behavior. Thousands of new neurons are added to the adult human hippocampus each day even in old age. Rodent studies have shown that immature neurons are more plastic than pre-existing neurons and while there is evidence that they make functional contributions to memory their exact role remains unclear. We are interested in the possibility that new neurons are important for learning under stress. It is well known that acute stress promotes hippocampal synaptic plasticity and learning, enabling us to remember our experiences and adapt our future behaviors accordingly. Furthermore, there is a growing body of work indicating that newborn neurons regulate emotional behaviors in response to stress. To test a stress-dependent function for new neurons in memory we trained neurogenesis-deficient GFAP-TK rats in the spatial water maze under high (16°C) or low (25°C) stress conditions. We find that, in male rats, blocking adult neurogenesis indeed impairs spatial learning and memory primarily at cold, stressful temperatures. Since males and females differ in stress reactivity, and little is known about functions for new neurons in males vs. females, we then examined how stress modulates learning and memory in female rats that lack neurogenesis. In contrast to males, neurogenesis-deficient female rats were not impaired when trained at 16°C, and even showed a trend for enhanced performance relative to their wild-type littermates. Furthermore, whereas temperature did not affect wild-type female rats, neurogenesis-deficient female rats performed significantly better and had greater corticosterone levels at 16°C than at 25°C. Collectively, our data indicate that adult neurogenesis regulates learning under stress and additionally suggests that new neurons perform distinct functions in the male vs. female brain.

Disclosures: T.P. O'Leary: None. **J.S. Snyder:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 725.19/BB28

Topic: F.02. Animal Cognition and Behavior

Support: R37 AG013622

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

David Geffen School of Medicine Dean's Fund

Title: A shared neural ensemble links distinct contextual memories encoded close in time

Authors: *D. J. CAI[1], D. AHARONI[2], T. SHUMAN[3], J. SHOBE[1], J. BIANE[5], J. LOU[3], I. KIM[1], K. BAUMGAERTEL[6], A. LEVENSTAIN[1], M. TUSZYNSKI[5], M. MAYFORD[6], P. GOLSHANI[3], A. J. SILVA[4];
[1]Neurobio., [2]Neurobiology, Neurology, Integrative Ctr. for Learning and Memory, [3]Neurology, Integrative Ctr. for Learning and Memory, Brain Res. Inst., [4]Neurobiology, Psychiatry, Psychology, Integrative Ctr. for Learning and Memory, Brain Res. Ins, UCLA, Los Angeles, CA; [5]Neurosciences, UCSD, La Jolla, CA; [6]Cell Biol. and Neurosciences, The Scripps Res. Inst., La Jolla, CA

Abstract: Recent studies suggest the hypothesis that a shared neural ensemble could link distinct memories encoded close in time. According to this hypothesis, one memory triggers a temporary increase in neuronal excitability that biases the representation of a subsequent memory to the same neuronal ensemble encoding the first memory, such that recall of one memory increases the likelihood of the recall of the second memory. Accordingly, using *in vivo* calcium imaging with a miniature fluorescent microscope and immediate early gene expression with TetTag transgenic mice, we found that the overlap between the hippocampal CA1 ensembles activated by two distinct contexts acquired within a day is higher than when the two contexts are separated by a week. Multiple convergent findings indicate that this overlap of neuronal ensembles links two contextual memories. First, fear paired with one context is transferred to a neutral context when the two are acquired within a day but not across a week. Second, the first memory strengthens the second memory within a day but not across a week. Older mice, known to have lower CA1 excitability, do not show the overlap between ensembles, the transfer of fear between contexts, or the strengthening of the second memory. Taken together, these findings demonstrate that contextual memories encoded close in time are linked by directing storage into overlapping ensembles. Alteration of these processes by aging could affect the temporal structure of memories, thus impairing efficient recall of related information.

Disclosures: **D.J. Cai:** None. **D. Aharoni:** None. **T. Shuman:** None. **J. Shobe:** None. **J. Biane:** None. **J. Lou:** None. **I. Kim:** None. **K. Baumgaertel:** None. **A. Levenstain:** None. **M. Tuszynski:** None. **M. Mayford:** None. **P. Golshani:** None. **A.J. Silva:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: FP7-ERC 'GABA Networks' grant (#242842)

DFG - 3657/1-1

WHRI CE4044U8WHRI0

Title: Functional organization of hippocampal microcircuits regarding the embryonic birthdate of neurons *in vivo*

Authors: *S. REICHINNEK[1], A. MALVACHE[2], V. VILLETTE[2], R. COSSART[2];
[1]Susanne Reichinnek, Marseille, France; [2]INMED, Marseille, France

Abstract: Cell type diversity classically reflects the available repertoire of neuronal functions in different brain regions. So far, cell diversity in the hippocampus was mainly addressed in GABAergic cells. In contrast, only few studies address the possibility that glutamatergic CA1 pyramidal cells may comprise several subtypes with different functional and morphological features (Thome 2014, Mizuseki 2011). We aim at understanding the relationship between the developmental temporal origin of glutamatergic neurons and their recruitment into behaviorally-relevant network dynamics. Using an inducible genetic fate mapping approach, we are able to label neurons depending on their embryonic birth date. Further on, we use calcium imaging (GCaMP6-M) to monitor their neuronal activity in head-fixed mouse voluntarily running on a treadmill using a 2-photon microscope. Monitoring hundreds of neurons in the dorsal hippocampus, we analyze their firing pattern during typical behaviorally relevant network oscillations e.g. theta-nested gamma and sharp wave ripples. We have probed the co-occurrence of calcium-related neuronal activity and the electrophysiological signature of the network oscillations using a contra-laterally implanted field electrode. We analyze the recruitment, the spatial organization and the inhibitory impact of neurons from different developmental ages. In this way, we investigate the functional organization of neurons born at different embryonic time-points during physiological network oscillations and provide insights into how developmental origin shapes the functional organization of hippocampal microcircuits.

Disclosures: S. Reichinnek: None. **A. Malvache:** None. **V. Villette:** None. **R. Cossart:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: Simons Foundation

NIH Grant EY020676

Title: Hippocampal replay correlates retrieval of fear memory in inhibitory avoidance task

Authors: *C.-T. WU[1], C. KEMERE[3], D. JI[2];
[1]Neurosci., [2]Baylor Col. of Med., Houston, TX; [3]Rice Univ., Houston, TX

Abstract: The hippocampus is critical for retrieval of contextual fear memory, but the patterns of neural activity that underlie this retrieval are largely unknown.We created a novel linear avoidance task to study the hippocampal activity underlying retrieval of fear memory. The 225-cm track comprises two distinct light and dark segments of equal length. Rats initially tend to remain in the dark segment. Following an initial exploration period, we shock the rats in the final 28-cm portion of the dark segment (shock zone). After being shocked, they completely avoid the shock zone and tend to remain in the light segment. We find that reactivation of place cells associated with shock zone precedes and is strongly correlated with avoidance behavior. This reactivation occurs during the replay of place cell sequences leading to or originating from the shock-zone and is associated with the high-frequency ripple oscillations. Hippocampal replay has been previously shown to predict future trajectories in goal-directed behavior. In contrast, the hippocampal replay we observe reflects a potential path that the animal strictly avoids presumably reflecting the negative affect of the shock experience. Our results suggest that hippocampal reactivation of place cell sequences provides salient spatial information associated with past memories for general decision making, but does not necessarily predict future actions. We also find that there is rate remapping with only small proportion of cells change their firing locations following an aversive experience.

Disclosures: C. Wu: None. **C. Kemere:** None. **D. Ji:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

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Topic: F.02. Animal Cognition and Behavior

Title: Anatomical localisation of episodic-like memory in the mouse

Authors: O. MONTEIRO, A. GUTOREVA, L. M. KAFER, R. C. KING, J. J. LAMBERT, *R. F. LANGSTON;
Div. of Neuroscience, Med. Res. Inst., Dundee, United Kingdom

Abstract: Distinct areas of the cortex and the hippocampus have been found to be important for associative recognition memory in the rat, depending on the level of processing of spatial and contextual information. Lesion studies found that the hippocampus is crucial for episodic-like memory in rats. Associative recognition memory tasks that incorporate novel object (what is new), location (where is this novelty) and context (which context has this novelty been encountered) is said to resemble human episodic memory. This episodic-like memory has not been characterised in mice. We have carried out spontaneous recognition memory tasks in mice that involve: object recognition (what), object-place recognition (where), object-context recognition (which) and object-place-context recognition (episodic-like memory). We examined activity-dependent immediate early gene expression (Fos) after mice had performed each of these behavioural tasks, in order to investigate differential activation of neurons in different hippocampal and cortical regions. The Fos gene is critical for learning and memory, and its region specific activation correlates with performance in a context discrimination task. Thus, Fos protein expression can be used as an indicator of neuronal activation in relation to specific behavioural paradigms. We will compare neural networks activated during each component of episodic memory to those found to be crucial in rats. This study will shed light on the neural circuitry involved in each component of episodic memory. In addition, most available disease models for neurodegenerative diseases and dementia are in mice. Hence, this study will identify crucial brain regions involved in the processing of episodic memory in a species more relevant for studying models of memory impairments.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC

NWO VENI grant 863.10.013

Title: Motivational state influences the content of hippocampal sequences

Authors: A. CAREY, *M. A. VAN DER MEER;
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Abstract: Internally generated sequences of hippocampal place cell activity, generated during sharp wave-ripple complexes (SWR), are thought to play a role in the consolidation and expression of hippocampus-dependent knowledge. The content of SWR-associated sequences emitted in awake states is not limited to literal "replay" of past experience: instead, they include biases towards goal locations (Pfeiffer & Foster, 2013), trajectories not recently experienced (Gupta et al. 2010), and even not-yet experienced sequences (Dragoi & Tonegawa, 2010). If the content of hippocampal sequences can be freed from literal experience, what factors determine their content? We hypothesized that shifts in preferred outcome influence the content of hippocampal sequences. To test this, rats (n = 4) performed a simple T-maze choice task, with one arm resulting in a 6% sucrose outcome, and the other in food pellet outcome. Animals were water-restricted or food-restricted on alternate days, such that they preferentially chose the water reward following water restriction and food reward following food restriction. We recorded ensembles of hippocampal place cells as animals performed the task, as well as during rest periods before and after the task. Overall, individual place cells were slightly more likely to participate in SWR events if they had a field on the sucrose arm compared to the food arm; importantly, however, there was no interaction with motivational state. Interestingly, a pairwise analysis revealed an interaction of activation probability with motivational state, such that pairs of cells with fields on the "water" arm were more likely to occur when water was preferred, and pairs of cells with fields on the "food" arm were more likely to occur when food was preferred. This interaction was significant even when only SWRs during the pre-task rest phase were included, and after the number of trials on either arm of the maze was matched to prevent biases in the estimation of place field locations. Next, we applied sequence detection methods to detect and classify multi-cell sequences as "water" or "food" depending on which arm of the maze they mapped onto. Individual animals tended to have idiosyncratic biases, but in animals where sequences could be reliably detected, motivational state shifted sequence content towards the preferred reinforcer. These results demonstrate that the content of hippocampal sequences is influenced by motivational state, and elucidate the complex relationship between experience and the content of memory.

Disclosures: A. Carey: None. **M.A. van der Meer:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: Medical Research Council UK, Career Development Fellowship to ASM G0800329

Wellcome Trust Career Development Fellowship to ACHL

Title: Neural plasticity in episodic memory: Functional and structural connectivity changes associated with learning and after fornix transection in macaques

Authors: *A. S. MITCHELL[1], D. J. MITCHELL[2], S. CHAKRABORTY[3], A. H. BELL[1,2], A. C. H. LEE[4,5];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]MRC Cognition and Brain Sci. Unit, Cambridge, United Kingdom; [3]Bioengineering, Imperial Col., London, United Kingdom; [4]Psychology (Scarborough), Univ. of Toronto, Toronto, ON, Canada; [5]Rotman Res. Inst., Toronto, ON, Canada

Abstract: Neuroimaging studies have documented changes in connectivity strength in the motor cortex during learning new motor skills. However, it is unknown how connectivity changes during learning episodic-like memories, and the impact on relearning after damage to structures implicated in episodic memory processing. We measured functional and structural changes using MR imaging in four macaques at multiple time points, initially during learning a visual cognitive task, and then during relearning after bilateral fornix lesions. We also collected neuroimaging in a matched control group that were not exposed to any form of learning. Naïve monkeys learnt an episodic-like memory task presented on a touchscreen monitor, which emphasized rapid within session learning of context specific object-in-place information for food rewards. Two identical 'bulls-eyes' (white background black centre) were used as the objects for all the discrimination problems. In each session, a monkey learnt by trial and error, which bulls-eye was the correct target by touching it. For each discrimination problem, the bulls-eyes were randomly located within a novel colourful visual background that filled the touchscreen. There were 10 novel discrimination problems, presented concurrently and repeated 8 times (i.e. 80 problems) per session. We collected whole brain functional (blood oxygenation level-dependent imaging) and structural (diffusion weighted imaging and structural T1-weighted imaging) data under general anaesthesia, prior to the start of learning and then after the monkeys had attained specific milestones in their learning. Following a pre-operative performance test and a final preoperative

scan, the monkeys received a fornix transection (there were 4 scans in total prior to surgery). An identical postoperative performance test was administered after 2 weeks of postoperative recovery and we collected a further four MR imaging datasets at regular intervals during post-surgery relearning. Behaviourally, as expected, monkeys learnt the task and, after fornix transection, their performance was markedly impaired. For the imaging data, we observed changes in functional connections (as measured by resting state correlations) and structural connections in extended hippocampal (medial temporal lobes and cingulate cortex) and prefrontal cortex regions associated with rapid within session learning of visual object-in-place information. These data suggest that cortical neural networks can reorganise at functional and structural levels during learning of an episodic-like memory task and after brain injury to a specific subcortical white matter structure.

Disclosures: **A.S. Mitchell:** None. **D.J. Mitchell:** None. **S. Chakraborty:** None. **A.H. Bell:** None. **A.C.H. Lee:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

RIKEN Brain Science Institute

Title: Activating positive memory engrams suppresses depression-like behavior

Authors: ***S. RAMIREZ**[1], X. LIU[3], C. J. MACDONALD[2], A. MOFFA[2], J. ZHOU[2], R. L. REDONDO[2], S. TONEGAWA[2];
[1]Brain and Cognitive Sciences, MIT, MIT, Cambridge, MA; [2]MIT, MIT, MA; [3]Northwestern Univ., Chicago, MA

Abstract: Stress is considered a potent environmental risk factor for many behavioral abnormalities, including anxiety and mood disorders. Animal models can exhibit limited but quantifiable behavioral impairments resulting from chronic stress, including deficits in motivation, abnormal responses to behavioral challenges, and anhedonia. The hippocampus is thought to negatively regulate the stress response and to mediate various cognitive and mnemonic aspects of stress-induced impairments though the neuronal underpinnings sufficient to

support behavioral improvements are largely unknown. Here, we acutely rescue stress-induced, depression-related behaviors by optogenetically reactivating DG cells that were previously active during a positive experience. A brain-wide histological investigation, coupled with pharmacological and projection-specific optogenetic blockade experiments, identified glutamatergic activity in the hippocampus-amygdala-nucleus accumbens pathway as a candidate circuit supporting the acute rescue. Finally, chronically reactivating hippocampal cells associated with a positive memory resulted in a rescue of stress-induced behavioral impairments and neurogenesis at time points beyond the light stimulation. Together, our data suggest that activating positive memories artificially is sufficient to suppress depression-like behaviors and point to DG engram cells as potential therapeutic nodes for intervening with maladaptive behavioral states.

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Poster

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Program#/Poster#: 725.26/BB35

Topic: F.02. Animal Cognition and Behavior

Title: Specific contribution of CA1 to the reconsolidation of contextual fear memory: an optogenetic/Arc molecular imaging study

Authors: ***V. LUX**[1], O. MASSECK[1], S. HERLITZE[2], M. M. SAUVAGE[2];
[1]Ruhr-Universitaet Bochum, Bochum, Germany; [2]Ruhr-Universität, Bochum, Germany

Abstract: Reactivation of a stored memory can lead to instability, necessitating the restabilization of the trace. This so-called reconsolidation process can be blocked using amnestic treatments following the reactivation of the memory. Thereby, the restabilization of the trace is thought to be affected, resulting in impaired memory performance when tested several hours later. While the basolateral amygdala (BLA) is known to play a crucial role in the reconsolidation process, less is known about the specific contribution of the hippocampus, especially that of the CA1 subfield, which has been reported to be critically involved in the consolidation and retrieval of fear memory and thus might also play a major role in the reconsolidation process. To investigate the role of CA1 in the reconsolidation of fear memory and evaluate the long lasting effects of a perturbation of this process on the activity of the 'consolidation network', we blocked the restabilization of memory by inhibiting cell firing in

CA1 using optogenetics and measured the effect of this manipulation on memory performance and the activity of CA1, CA3 and the BLA upon reexposure to the conditioning context the following day. Inhibiting cell firing of CA1 following reactivation of the fear memory trace led to a significant decrease in freezing behavior when mice were exposed to the conditioning box the following day. Moreover, using a high resolution molecular imaging technique based on the detection of the immediate-early gene Arc, we could show for the first time that this memory impairment was paralleled by a reduction in cellular activity in CA1, CA3 and the BLA. Thus, our results suggest that CA1 might play an important role in the reconsolidation of contextual fear memory since blockade of cell firing in CA1 upon reactivation of the memory trace results in both behavioral and cellular effects at a network level.

Disclosures: **V. Lux:** None. **O. Masseck:** None. **S. Herlitze:** None. **M.M. Sauvage:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: FP7-ERC 'GABA Networks' grant (#242842)

Title: Imaging the spatio-temporal organization of "replay" in the CA1 region of awake mice

Authors: ***A. MALVACHE**, V. VILLETTE, S. REICHINNEK, R. COSSART;
INMED, INSERM U901, Marseille, France

Abstract: Sequential activation of hippocampal neurons has been shown to carry information about current, past or future locations. These sequences can be generated by external cues, as reported for the sequential exploration of place fields, as well as internally, for example during the delay period of a memory task. Sequences formed during spatial exploration have been shown to be re-activated at a shorter timescale during voluntary rest and sleep. This "replay" occurs during ripple events in the hippocampus. Here, we have studied awake replay using two-photon calcium imaging of neuronal activation in the CA1 region of the dorsal mouse hippocampus. Mice are head fixed but allowed to travel on a self-paced treadmill. We have previously shown that during run epochs, in the absence of external cues, CA1 neurons in the pyramidal layer are sequentially activated and that these sequences integrate the distance travelled by the mouse. During rest epochs, we have imaged the partial replay of these sequences that clustered into well-defined groups. A large fraction of such events occurred together with

ripple events recorded electro-physiologically from the contralateral CA1 hippocampus. We took advantage of chronic imaging to study the spatial patterning and evolution of replay events across days.

Disclosures: **A. Malvache:** None. **V. Villette:** None. **S. Reichinnek:** None. **R. Cossart:** None.

Poster

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NSF IIS-1302256

Title: Systematic data mining of hippocampal synaptic properties

Authors: *K. MORADI, C. L. REES, D. W. WHEELER, A. O. KOMENDANTOV, C. M. WHITE, D. J. HAMILTON, S. VENKADESH, M. SULIMAN, G. A. ASCOLI;
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Abstract: The synaptic properties of the rodent hippocampal formation have been extensively investigated for several decades. The amplitude, kinetics, and (short- and long-term) plasticity of excitatory and inhibitory potentials depend both on the type of pre- and post-synaptic neurons, and vary substantially among regions (e.g. dentate gyrus, CA3, CA1, and entorhinal cortex). While certain connections are better characterized (e.g. the Schaffer collateral from CA3 pyramidal to CA1 pyramidal cells), the lack of a systematic accounting of published synaptic data prevents a comprehensive comparison across pairs of neuron types. Hippocampome.org, a knowledge base that identified 122 neuron types based on morphological, electrophysiological, and molecular evidence, enables integration and dense coverage of the available synaptic data. The anatomical distribution of axons and dendrites exposes more than 3200 “potential connection” among neuron types (“Peters’ Rule”). Extensive literature mining indicates that fewer than 10% of these potential connections have been tested experimentally to date. Among those, synapses have been confirmed in approximately five-out-of-six neuron type pairs.

However, less than 50% of these known connections have been characterized electrophysiologically in peer-reviewed publications. In these cases, we are extracting information about synaptic amplitude, kinetics, and, when available, short-term and long-term plasticity. Due to widely non-uniform experimental methods and conditions, these data must be normalized to enable meaningful quantification. **Keywords**: microcircuits, neural networks, connectivity, neuroinformatics, physiology, neuron types

Disclosures: **K. Moradi:** None. **C.L. Rees:** None. **D.W. Wheeler:** None. **A.O. Komendantov:** None. **C.M. White:** None. **D.J. Hamilton:** None. **S. Venkadesh:** None. **M. Suliman:** None. **G.A. Ascoli:** None.

Poster

725. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 725.29/BB38

Topic: F.02. Animal Cognition and Behavior

Support: Deutsche Forschungsgemeinschaft (SFB1089)

Title: The firing of medial septal PV+ interneurons reduces the population activity of CA1 pyramidal neurons - potential microcircuit mechanisms

Authors: ***D. JUSTUS**[1], F. FUHRMANN[1], L. SOSULINA[1], H. KANEKO[1], C. HANNES[1], T. BEUTEL[1], D. FRIEDRICHS[1], S. SCHOCH[2], M. FUHRMANN[1], S. REMY[1,3];
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Abstract: During locomotion the hippocampal formation undergoes a transition to a state specialized for the processing of spatial information. This brain state is associated with strong field potential oscillations in the theta band (5-12 Hz). The medial septal nucleus and the diagonal band of Broca (MSDB) are known to be major pacemakers of hippocampal theta oscillations. Using fluorometric monitoring of GCaMP6 expressed exclusively in MSDB parvalbumin positive (PV+) interneurons, we showed that this cell-type increases its activity during locomotion. Expressing ChR2-EYFP (H134R) MSDB PV+ interneurons allowed optogenetic activation of these neurons at theta band frequencies. We observed that hippocampal LFP oscillations can be reliably driven by the firing of PV+ interneurons in the medial septum. Interestingly, using whole-cell patch-clamp recordings in awake mice, an increased synchrony of both subthreshold oscillations and action potential output could also be found on the level of

single CA1 pyramidal neurons. Rhythmic stimulation of glutamatergic VGluT2+ neurons in the MSDB had similar effects on LFP oscillations and synchrony of action potential output, consistent with a strong intraseptal interconnectivity of these neurons. However, following blockade of glutamatergic transmission locally in the MSBD with NBQX and D-AP5, the correlation of hippocampal theta frequency and locomotion velocity was strongly impaired and the amplitude of LFP oscillations induced by VGluT2 stimulation was strongly reduced. This finding confirmed a crucial role of PV+ interneurons and PV+ septo-hippocampal projections for the hippocampal theta rhythm. Two-photon imaging of GCAMP6 expressing CA1 pyramidal neurons through a cranial window in awake, head-fixed mice on a treadmill revealed a second interesting function of MSDB PV+ interneurons: Their firing reduced the activity of hippocampal principal cell population by about 25 %. This finding could be confirmed on the single cell level using in-vivo whole-cell patch-clamp recordings. Thus MSDB PV+ interneurons might be important regulators of CA1 population activity during locomotion. This effect could be mediated via an intraseptal inhibition of VGluT2+ neurons or a PV+ mediated regulation of CA1 microcircuits via septo-hippocampal projections.

Disclosures: D. Justus: None. **F. Fuhrmann:** None. **L. Sosulina:** None. **H. Kaneko:** None. **C. Hannes:** None. **T. Beutel:** None. **D. Friedrichs:** None. **S. Schoch:** None. **M. Fuhrmann:** None. **S. Remy:** None.

Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 726.01/BB39

Topic: F.02. Animal Cognition and Behavior

Support: R01 HD029421-18

Title: Postnatal choline supplementation ameliorates long-term disruptions in behavior and hippocampal gene expression resulting from fetal iron deficiency

Authors: B. C. KENNEDY[1], M. KOHLI[2], J. MAERTENS[2], M. T. PISANSKY[1], P. V. TRAN[3], *J. C. GEWIRTZ[2], M. K. GEORGIEFF[3];
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Abstract: Iron deficiency during late fetal and early postnatal life can lead to persistent deficits in cognitive function. In rodent models these changes are accompanied by long-term disruptions in expression of hippocampal synaptic plasticity genes, which likely contribute to the poor

spatial and recognition memory observed in formerly iron deficient (FID) rats and mice. When given prenatally from embryonic day (E) 11-18, choline supplementation improves memory and normalizes hippocampal gene expression in adult FID rats. However, this treatment is translationally limited by the difficulty of assessing fetal iron status during prenatal development. Although choline supplementation given postnatally has also been shown to improve cognitive performance in rodents, this approach has not been assessed in iron deficiency. Iron-deficient (6ppm) or iron-sufficient (200 ppm) diet was provided to rats from E3 to postnatal day (P) 7. Half of the resulting offspring in each group were given choline supplementation from P11-30, for a total of four treatment groups: formerly iron deficient (FID), formerly iron deficient with choline supplementation (FID-C), iron sufficient (IS), and iron sufficient with choline supplementation (IS-C). The effects of gestational and postnatal diet on adult behaviors were assessed using novel object recognition (NOR), Barnes maze, and pre-pulse inhibition (PPI) tasks. In separate groups of adult animals, hippocampi were extracted and processed for analysis of gene expression using qRT-PCR. FID rats exhibited poor working memory in the Barnes maze and trended toward impaired recognition memory and PPI relative to IS controls. Postnatal choline supplementation of the FID-C group improved performance in working memory, NOR and PPI. However, choline supplementation of the IS-C group resulted in impaired reversal learning and working memory in the Barnes maze. Early life iron deficiency also disrupted expression of hippocampal synaptic plasticity genes relative to IS controls. Expression of many of the affected genes was normalized by postnatal choline supplementation. Consistent with the behavioral findings, choline supplementation itself dysregulated some genes in the IS-C hippocampus, suggesting an iron status-dependent effect of postnatal choline supplementation on hippocampal function and memory. Overall, postnatal choline supplementation could hold promise for mitigating the long-term effects of early life iron deficiency but may be detrimental when given to IS individuals.

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Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

Location: Hall A

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Topic: F.02. Animal Cognition and Behavior

Support: Grants-in-Aid for Scientific Research on Priority Areas “Molecular Brain Science” from the Ministry of Education, Culture, Sports, Science and Technology of Japan(Grant Number 20022012)

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Asahi Glass Foundation

Sumitomo Foundation

Title: SCOP mediated circadian regulation of recognition memory

Authors: *K. SHIMIZU, Y. FUKADA;
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Abstract: Previous studies demonstrate that diurnal rhythm affects memory formation. However it is not yet clear if the internal clock regulates the efficiency of the memory formation, and there is no molecular-based evidence that connects the memory formation and circadian rhythms. Novel object recognition tests were performed with adult male mice over the circadian time. Long-term memory efficiency varied in a circadian manner and hence it seems to be controlled by the endogenous circadian clock. In fact, electrolytic lesion of the suprachiasmatic nucleus (SCN), the master circadian clock, disrupted the circadian rhythm of long-term memory formation. We are focusing on SCOP and related molecules to find molecular processes that connect the circadian clock with memory formation. SCOP is expressed in a circadian manner in the mouse SCN, and SCOP negatively regulates K-Ras function and its downstream ERK/MAPK pathway. In the hippocampus, SCOP is indispensable for long-term memory formation for novel objects. These data together suggest that SCOP-ERK pathway plays a key role for the circadian control of long-term memory formation in the hippocampus. SCOP knockdown by shRNA expression lentivirus or SCOP knockout in the hippocampus attenuated circadian oscillation of the long-term memory for novel objects. These data suggest that circadian regulation of long-term memory formation for novel objects is dependent on SCOP protein in the hippocampal neurons.

Disclosures: **K. Shimizu:** None. **Y. Fukada:** None.

Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

Location: Hall A

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Program#/Poster#: 726.03/BB41

Topic: F.02. Animal Cognition and Behavior

Title: Diabetes affects adversely and differentially the hippocampal neurogenesis during pregnancy and non-pregnancy period in young rats

Authors: *M. S. RAO, S. SHIVANANDAN, A. M. SONY;
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Abstract: The pregnancy period is one of the most plastic periods in a female's life. During this period many physiological, hormonal, cellular and molecular changes happen in the female body systems and nervous system which prepare the female for the challenges of motherhood. Certain neural mechanisms, neuronal plasticity, neurogenesis are affected differently during pregnancy and non-pregnancy physiological conditions. Hippocampal adult neurogenesis has been shown to increase during later part of pregnancy and decrease after delivery through lactation period in rats. Diabetes mellitus, a disease characterized by increased level of glucose in the blood, known to affect the hippocampal neurogenesis. Although literature indicated the deleterious effects of diabetes on developing fetus, there are only a few reports on its effects on the hippocampal dentate gyrus(DG) neurogenesis in pregnant women and experimental animals. The objective of the present experiment was to study the effects of diabetes during pregnancy and non-pregnancy period on hippocampal dentate gyrus (DG) neurogenesis. Adult pregnant female Wistar rats (4 months old) were divided into two groups(n=6 rats in each group). i). Normal pregnant rats(NP) - remained without any treatment till delivery, ii) Diabetic pregnant rats(DP)- Diabetes was induced in these pregnant rats by injecting streptozotocin (50mg/kg)intravenously through dorsal tail vein on 10^{th} day of gestation. Blood glucose level was measured to confirm the diabetic condition 48 hrs after STZ injection. They were maintained in sterile cages till delivery. After delivery rat pups were discarded and dams in both groups and age matched normal control (NC) and Diabetic Control (DC) rats were anesthetized with halothane, perfused transcardially with saline followed by 4% paraformaldehyde, brain was dissected and processed for frozen sections. Sections(30µm) were immunostained with doublecortin (DCX) to analyze the neurogenesis. Data were analyzed with one way ANOVA followed by Bonferroni's test. Results showed significantly decreased DG neurogenesis in DC group compared to NC($P<0.001$). Number of DCX positive neurons in NP rat was significantly more compared to DP group ($P<0.001$), however it was significantly increased compared to NC ($P<0.001$). Comparison of neurogenesis between DC and DP, DP showed significantly less new neurons ($P<0.01$). Results of our experiment suggests that, diabetes affects neurogenesis differentially during pregnancy and non-pregnancy period. We conclude that diabetes affects the neurogenesis adversely in the pregnant female rats, which may affect the their maternal behavior

Disclosures: M.S. Rao: None. **S. Shivanandan:** None. **A.M. Sony:** None.

Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

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Program#/Poster#: 726.04/BB42

Topic: F.02. Animal Cognition and Behavior

Support: NASA NNX07AP84G

NASA NNX12AB55G

NIH DA 016765

NIH DA 023555

NIH DA 007290

Title: What happens to mouse hippocampal-dependent behavior and neurogenesis on the way to Mars? One small step for mousekind..

Authors: *A. J. EISCH[1], M. J. LUCERO[1], R. L. REDFIELD[1], N. ITO[1], D. R. RICHARDSON[1], R. P. REYNOLDS[1], G. PALCHIK[2], S. MUKHERJEE[1], A. K. WALKER[1], C. W. WHOOLERY[1], H.-Y. SHIH[2], P. D. RIVERA[1], S. G. BIRNBAUM[1], B. P. C. CHEN[2]; [1]Dept Psychiatry, [2]Dept Rad Oncology, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Space radiation consists of high atomic weight and energy (HZE) particles. Chronic low doses of HZE particles - as will be encountered on deep space missions, such as to Mars - may be detrimental to astronauts by diminishing hippocampal function and thus may compromise mission success. Studies using ground-based space radiation and relatively young adult mice (2 months of age) show that HZE particles decrease hippocampal neurogenesis and performance on hippocampal-dependent tasks. However, it is unknown how HZE radiation influences the brain and behavior of “astronaut age” equivalent mice (6 months of age), or how it alters important hippocampal functions such as contextual discrimination. To fill these knowledge gaps, C57BL/6J mice (9-week old [Young Adult] or ~6-month-old [Mature] mice) were exposed to HZE particles (28Si, 275 MeV/n, 72.1 KeV/μm LET of a single exposure of 20 cGy, 100 cGy, or 0 cGy [Sham] or 56Fe, 600 MeV/n, 174.1 KeV/μm LET of a single exposure of 20 cGy [Non-fractionated], protracted dose of 20 cGy [Fractionated, 6.7 cGy x 3 days, 48h intervals], or 0 cGy [Sham]). General behavior (e.g. locomotion) and 2 hippocampal-dependent functions, contextual fear conditioning (CFC) and contextual discrimination fear conditioning (CDFC) were studied 2 to 6 months post-irradiation (IRR). After 28Si or 56Fe IRR, Young

Adult mice showed normal general behavior, but dose-dependent changes in CFC and CDFC performance. In contrast, while Mature mice displayed normal locomotor activity and normal CFC, they also showed a dose-dependent increase in contextual discrimination ability (CDFC) compared to Sham. The improvement in CDFC in Mature mice was unexpectedly not correlated with the number of new dentate gyrus neurons. Thus, HZE particle radiation unexpectedly enhances contextual discrimination in astronaut-age equivalent mice. We are currently investigating regulatory mechanisms underlying the age-related improvement in contextual discrimination after HZE radiation exposure, including the potential role of altered dentate gyrus neural circuitry and inhibitory tone, and the relationship between improvement in contextual discrimination and performance on other mission-relevant behavioral tasks.

Disclosures: **A.J. Eisch:** A. Employment/Salary (full or part-time):; Ut Southwestern Medical Center. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NASA, NIH. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH, NASA. **M.J. Lucero:** None. **R.L. Redfield:** None. **N. Ito:** None. **D.R. Richardson:** None. **R.P. Reynolds:** None. **G. Palchik:** None. **S. Mukherjee:** None. **A.K. Walker:** None. **C.W. Whoolery:** None. **H. Shih:** None. **P.D. Rivera:** None. **S.G. Birnbaum:** None. **B.P.C. Chen:** None.

Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

Location: Hall A

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NIH MH107945

NASA NNX12AB55G

NASA NNX15AE09G

Title: Image-guided cranial irradiation-induced ablation of dentate gyrus neurogenesis diminishes extinction of young - but not old - morphine reward memories

Authors: *M. L. MENDOZA, P. D. RIVERA, R. P. REYNOLDS, A. L. JUST, S. G. BIRNBAUM, A. J. EISCH;
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Abstract: Dentate gyrus adult neurogenesis is implicated in many hippocampal-dependent functions. However, the role of adult neurogenesis in context-dependent memory is unclear, as conflicting data exist on the role for them in retrieval and extinction of fear-associated memories. In addition, the role of adult neurogenesis in performance on reward-based context-dependent paradigms, like conditioned place preference (CPP), is particularly understudied. Here we used image-guided, hippocampal-targeted X-ray irradiation (IG-IR, 15 Gy) and morphine CPP to explore whether the dentate gyrus and in particular adult neurogenesis play a role in the retrieval or extinction of young and older reward memories in adult C57BL/6J male mice. Six weeks post-irradiation or Sham treatment, mice underwent morphine CPP (Pretest, Day 1; Pairing, Days 2-4 [saline AM, morphine PM, 7 or 15 mg/kg s.c.]). In keeping with prior work in cocaine CPP in rat, retrieval of a young memory of the morphine-paired chamber (Test, Day 5, 24h post-CPP) was similar in IG-IR and Sham mice. In addition, retrieval of an older memory (Test, Day 21, 3 weeks post-CPP) was also similar in IG-IR and Sham mice. However, extinction of a young memory of the morphine-paired chamber (Test, Days 5-24) was significantly and strikingly diminished in IG-IR mice compared to Sham mice. For example, while Sham mice extinguished a young drug-associated context reward memory in 6 days, IG-IR mice took more than twice as long (14 days). In contrast, extinction of an older memory (Test, Days 25-44) was roughly similar in IG-IR and Sham mice. Taken together, these data show that hippocampal-directed irradiation and the associated decrease in dentate gyrus adult neurogenesis interfere with extinction of young context-dependent reward memories. This work suggests that a reduction or loss of adult DG neurogenesis in humans may trigger or perpetuate the cycle of addiction.

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Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

Location: Hall A

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Program#/Poster#: 726.06/BB44

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01 MH102595

Title: Opposite effects of behavioral experience on zif268 expression in mature and immature adult-born hippocampal neurons

Authors: *K. A. HUCKLEBERRY, G. KANE, R. MATHIS, S. COOK, M. R. DREW;
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Abstract: The dentate gyrus is one of the few brain regions that generates new neurons throughout adulthood. To investigate the contribution of adult-born neurons to hippocampal function, numerous studies have characterized the effects of behavioral manipulations on immediate early gene (IEG) expression in adult-born neurons. These studies suggest that adult-born neurons integrate into hippocampal circuits and are recruited into networks supporting hippocampus-dependent memory. However, studies of IEG expression in adult-born neurons have typically used c-Fos or Arc, which are expressed at very low levels in neurons less than about 4 weeks of age. Here we characterize behavior-evoked IEG expression using zif268, which is expressed robustly in immature neurons (Snyder et al., 2008). We first quantified zif268 expression in doublecortin-positive (DCX+) immature neurons and in the general granule cell population after brief exposure to a novel environment. In the general granule cell population, zif268 expression peaked 1 hour after novel environment exposure and returned to baseline by 8 hours post-exposure. However, in the DCX+ cells, zif268 expression was suppressed relative to home cage for at least 8 hours post-exposure. We next asked whether suppression of zif268 in DCX+ immature cells occurs in other behavioral paradigms that recruit the hippocampus. Exposure to Morris water maze training, an enriched environment, or a novel environment caused approximately equal suppression of zif268 expression in DCX+ cells and approximately equal activation of zif268 expression among the general granule cell population. The same behavioral procedures activated zif268 expression in 6-week-old BrdU-labeled adult-born neurons, indicating that zif268 suppression is specific to immature neurons. Finally, we asked whether zif268 suppression varied as a function of age within the DCX+ population, which ranges in age from 0 to approximately 4 weeks. Novel environment exposure had no significant effect on zif268 expression in 2- or 4-week-old BrdU-labeled neurons, but it significantly suppressed zif268 expression in 3-week-old neurons. In summary, behavioral experience transiently activated expression of zif268 in mature granule cells but caused a lasting suppression of zif268 expression in immature, adult-born granule cells. We hypothesize that zif268 suppression could function to prevent neurons from undergoing learning-related synaptic plasticity while they are immature or to support learning-induced apoptosis.

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Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 726.07/BB45

Topic: F.02. Animal Cognition and Behavior

Title: Behavioral alterations following combined binge alcohol and nicotine exposure in adult rats: An analysis of spatial learning and memory

Authors: R. T. LINGG, A. M. FORMICA, A. C. ROCCAFORTE, *D. M. HAYES; Radford Univ., Radford, VA

Abstract: Excessive alcohol consumption has consistently been associated with drastic impairments in neurological functioning ranging from structural to complex behavioral abnormalities, but the long term cognitive effects of nicotine use are less clearly established. Within the adult brain, researchers have shown impairments from alcohol and nicotine abuse to specific components of adult neurogenesis. Specifically, research has shown that both binge alcohol consumption and chronic nicotine abuse result in a substantial depression of cell proliferation within the subgranular zone (SGZ) of the dentate gyrus of the hippocampus; an area associated with ongoing plastic developments involved in spatial learning and memory. Importantly, reduced neurogenic activity has been hypothesized to be a potential mechanism underlying alterations in spatial learning and memory performance, in addition to a modulation of affective states. As alcohol and nicotine are the most commonly co-abused substances, further elaboration regarding the behavioral correlates of their combined use is critical. To that end, adult male Sprague-Dawley rats were injected with nicotine (0.3 mg/kg in 0.9% saline) or vehicle every 8 hours for 10 days. For the final four days of exposure, rats also received intragastric intubations of an ethanol-containing diet (25% w/v in Vanilla Ensure Plus®) or control diet thrice daily (mean dose: 6.35 ± 0.31 g/kg/day). Following extinction of acute withdrawal behaviors (~18hrs) spatial learning and memory, in addition to thigmotaxis, were assessed using a Morris Water Maze task (Days 5 - 30 post-administration). A significant impairment, attributable to nicotine exposure, was observed in latency and swim distance required to reach the submerged platform. Further, nicotine administration was associated with an increased prevalence of anxiety-related behaviors during the initial acquisition phase. Ethanol, however, was not found to influence learning or thigmotaxis; suggesting that as early as five days post exposure, the effects of ethanol on behavioral functioning are negligible. Interestingly, dual ethanol and nicotine treatment attenuated the nicotine-induced learning impairment during an analysis of cognitive flexibility, potentially a result of the anxiolytic effects of ethanol. Thus, the pattern of inhibited neurogenic action produced by chronic exposure to nicotine may be

implicated in adverse behavioral outcomes, as evidenced by impaired spatial learning and memory performance.

Disclosures: **R.T. Lingg:** None. **A.M. Formica:** None. **A.C. Roccaforte:** None. **D.M. Hayes:** None.

Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

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Program#/Poster#: 726.08/BB46

Topic: F.02. Animal Cognition and Behavior

Support: Italian Ministry of Health RF2009-1543811

Title: Effects of extremely low-frequency electromagnetic fields on olfactory memory in mice: role of increased neurogenesis and characterization of underlying molecular mechanisms

Authors: *A. MASTRODONATO[1], S. A. BARBATI[1], L. LEONE[1], C. COLUSSI[2], M. V. PODDA[1], C. GRASSI[1];
[1]Inst. of Human Physiol., Univ. Cattolica Med. Sch., Rome, Italy; [2]Inst. of Cell Biol. and Neurobio., Rome, Italy

Abstract: We recently demonstrated that exposure to extremely low-frequency electromagnetic fields (ELFEF) enhances hippocampal-dependent spatial learning and memory by increasing hippocampal neurogenesis (Leone at al., 2014). Aim of the present study was to investigate whether ELFEF stimulation also affects olfactory memory by modulating neurogenesis in the subventricular zone (SVZ) of the lateral ventricle. To address this issue we first performed behavioral tests on control (sham-) and ELFEF-exposed mice. We found that 30 days after the completion of ELFEF stimulation protocol (1 mT; 50 Hz; 3.5 h/day for 12 days) ELFEF-exposed mice showed a higher discrimination index between a familiar and a novel odor than controls (82.8 % and 61.3 %, respectively; $n = 8$; $p<0.05$). Sixty minutes after the first trial, animals were exposed again to the same odor for 5 minutes: ELFEF-exposed mice showed a significant decrease in the time of exploration on the second presentation than controls (4.3 ± 0.3 vs 8.1 ± 1.3 s, respectively; $p<0.05$) and an increased digging time near the odor previously rewarded (21.3 ± 0.4 vs 16.8 ± 3.9 s; $p<0.05$). Interestingly, immunohistochemical analyses performed in ELFEF-exposed mice revealed an increase in neural stem cell (NSC) proliferation, as assessed by the number of BrdU+ incorporating cells ($3,735 \pm 25$ vs $2,010 \pm 68$ in controls; $n = 3$ mice; $p<0.05$). The number of newborn NSCs differentiating towards the neuronal phenotype (i.e.,

BrdU+/DCX+ double-labeled cells) were similarly increased in the SVZ of ELFEF-exposed mice (3,303 ± 153 vs 1,746 ± 100 cells in controls; n = 3; $p<0.05$). These effects were associated with upregulated expression of mRNA encoding for the proliferation and pro-neuronal transcription factors Hes1, Mash1 and Wnt3, assessed by real-time PCR. To identify the molecular mechanisms underlying ELFEFs' effects, we extended our studies to an *in vitro* model of NSCs isolated from the SVZ of newborn mice. We focused our molecular analyses on Wnt/β-catenin signaling as critical regulators of adult SVZ neurogenesis. Real-time PCR showed a significant increase of Wnt3 mRNA in ELFEF-exposed NSCs. More importantly, upregulation of Wnt3 expression was associated with longer lasting expression of the transcription factor β-catenin in the nucleus of ELFEF-exposed NSCs. Accordingly, both Western blotting and immunofluorescence confocal microscopy revealed increased levels of β-catenin expression in the nuclei of ELFEF-exposed NSCs. Collectively, our findings suggest that ELFEF stimulation increases olfactory memory probably via enhanced Wnt/β-catenin signaling in the SVZ.

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Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 726.09/BB47

Topic: F.02. Animal Cognition and Behavior

Support: Abbott CNLM ZA70

Title: Chemotherapy impairs cognitive performance and reduces neurogenesis in mice, independent of nutritional intervention

Authors: ***A. SHERIFF**[1], T. K. BHATHACHARYA[2], A. COBERT[3], C. RENDEIRO[2], H. CHEN[4], E. J. ROY[5], W. G. HELFERICH[4], J. S. RHODES[6];
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Abstract: "Chemobrain" refers to long-lasting deficits in cognitive performance resulting from chemotherapy. Nonetheless, objective evidence for the existence of chemobrain is still lacking and the underlying mechanisms are debatable. One leading hypothesis is that the chemotherapeutic agents cross the blood-brain barrier and reduce the progenitor cell population in the hippocampus, a critical region for learning and memory that continues to generate new

neurons throughout life. The purpose of this study was to first determine whether a reliable behavioral deficit can be found in mice in response to administration of the chemotherapeutic agents standardly used to treat breast cancer in humans. Secondly, we aimed to address whether a nutritional intervention containing fish oil rich in omega-3 & 6 fatty acids could ameliorate those deficits in association with increased adult hippocampal neurogenesis. Mice received two doses of the chemotherapy treatment, specifically doxorubicin (IV, 4mg/kg), cyclophosphamide (IP, 80mg/kg) and 5-fluorauracil (IP, 40mg/kg) and were also injected with bromodeoxyuridine (BrdU, 50mg/kg) to label dividing cells. Following recovery from the chemotherapy, mice received intervention diets containing fish oil rich in omega-3 & 6 fatty acids or a standard control diet. Locomotor activity in the home cage and rotarod performance were assessed as well as learning and memory performance, as measured by Morris water maze (MWM), Y-maze and novel odor recognition. Our results show a significant spatial learning impairment in the MWM as a result of chemotherapy, specifically 2.5 months after treatment. This was also accompanied by a significant reduction in hippocampal neurogenesis in the chemotherapy animals. No changes in motor performance or locomotion were detected. On the other hand, supplementation with omega 3 and 6 enriched fish oil did not rescue the chemotherapy-induced deficits in learning or neurogenesis. Overall, our results strongly suggest a long-term effect of chemotherapy on both behavioral and neurological measures. As such, the present chemotherapy model might be useful in future studies to investigate dietary strategies or other types of interventions aimed at ameliorating chemotherapy cognitive impairments.

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Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

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Topic: F.02. Animal Cognition and Behavior

Support: CNRS grant (Lebanon)

Title: Thalamic stimulation in awake rats induces neurogenesis in the hippocampal formation

Authors: F. CHAMAA, W. SWEIDAN, Z. NAHAS, N. SAADE, *W. ABOU-KHEIR; American Univ. of Beirut, Beirut, Lebanon

Abstract: Background: Deep brain stimulation (DBS) provides substantial clinical benefits for a variety of movement disorders and lately emerged as a potential treatment for cognitive and mood disorders. Regulation of adult hippocampal neurogenesis may play a chief role in mediating DBS effects. Objective: To investigate the effects of unilateral anteromedial thalamic nucleus (AMN) stimulation on adult hippocampal neurogenesis in awake and unrestrained rats. Methods: Four groups of adult Sprague-Dawley male and female rats received unilateral stimulation (n=6 each) or sham surgery of electrode implantation with no current delivery (n=4 each) in the right AMN; another group of males (n=4) was stimulated in the right ventral posterolateral thalamic nucleus (VPL). A naïve group of males and females (n=4 each) was also included. Rats received 4 injections (50mg/Kg/injection) of 5'-bromo-2'-deoxyuridine (BrdU) 3 days post-surgery and were euthanized 24h later. The fractionator method was used together with confocal immunofluorescent analysis to probe for BrdU-, GFAP- and NeuN-positive cells in the dentate gyrus (DG) and hilar zone of the hippocampus. Results: Focal neurogenesis was induced in the ipsilateral DG after AMN and not VPL stimulation. Stimulation-induced effects were gender-independent and translated into a 76% increase in proliferation of neural stem/progenitor cells. Increased neurogenesis was most prominent at the caudal region of the DG, while no effect was detected in the hilar and the subventricular zones. Conclusions: The current study presents an exclusive hippocampal neurogenic response to AMN and not VPL stimulation; thereby suggesting the possible involvement of the components of Papez circuitry in mediating DBS effects and in the treatment of cognitive and behavioral disorders.

Disclosures: F. Chamaa: None. **W. Sweidan:** None. **Z. Nahas:** None. **N. Saade:** None. **W. Abou-Kheir:** None.

Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: FA2010

FRC2009

APRRTT2010

Title: Genetic manipulation of adult-born hippocampal neurons rescues memory in a mouse model of Alzheimer's disease

Authors: *C. RAMPON[1], K. RICHETIN[1], T. ANDRAINI[1], M. MOULIS[2], N. TONI[3], T. GALLOPIN[4], L. ROYBON[5], P. BELENGUER[2], M.-C. MIQUEL[2];
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Abstract: In mouse models of Alzheimer's disease, neurogenesis is impaired and the granule neurons that are generated fail to integrate existing networks. As new granule neurons participate to memory capacities, we hypothesized that enhancing neurogenesis should improve functional plasticity in the hippocampus and restore cognitive deficits in these mice. Thus, we performed a screen of transcription factors that could potentially enhance adult hippocampal neurogenesis. We identified Neurod1 as a robust neuronal determinant with the capability to direct hippocampal progenitors towards an exclusive granule neuron fate. Importantly, Neurod1 also accelerated neuronal maturation and functional integration of new neurons. When tested in an APPxPS1 mouse model of Alzheimer's disease, directed expression of Neurod1 in cycling hippocampal progenitors conspicuously reduced dendritic spine density deficits on new hippocampal neurons, to the same level as that observed in healthy age-matched control animals. Remarkably, this population of highly connected new neurons was sufficient to restore spatial memory in these diseased mice. In order to get insights into the mechanisms triggered by Neurod1, we focused on mitochondria, which functions are deeply impaired in neurodegenerative diseases such as Alzheimer's disease. We examined the contribution of mitochondria to neuritic maturation and spinogenesis in primary neurons and determined the impact of targeted expression of Neurod1 on the mitochondrial content of hippocampal adult-born neurons in our mouse model of AD.

Disclosures: C. Rampon: None. **K. Richetin:** None. **T. Andraini:** None. **M. Moulis:** None. **N. Toni:** None. **T. Gallopin:** None. **L. Roybon:** None. **P. Belenguer:** None. **M. Miquel:** None.

Poster

726. Learning and Memory: Genes, Signaling, and Neurogenesis II

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant MH038752

NIMH Grant MH090236

NIMH Grant MH095380

NIMH Grant MH104656

Title: GSK3β isoform-selective regulation of depression-like behavior, novel object recognition and hippocampal neural precursor cell proliferation

Authors: *M. PARDO, E. ABRIAL, R. JOPE, E. BEUREL;
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Abstract: Abnormally active glycogen synthase kinase-3 (GSK3) has been demonstrated to contribute to multiple pathological processes in mice, such as increasing susceptibility to dysregulation of mood-relevant behaviors, impairing performance in several cognitive tasks, and impairing adult hippocampal neural precursor cell proliferation. These deficits are all evident in GSK3α/β knockin mice, in which serine-to-alanine mutations block the inhibitory serine phosphorylation regulation of both GSK3 isoforms, leaving GSK3 hyperactive. In order to test if either GSK3 isoform has a predominant effect in each of these pathologies, here we examined these characteristics in GSK3α knockin mice and GSK3β knockin mice in which only one isoform was mutated. Only GSK3β, not GSK3α, knockin mice displayed heightened vulnerability to the learned helplessness model of depression-like behavior. Three cognitive measures that are impaired in GSK3α/β knockin mice demonstrated differential regulation by GSK3 isoforms. Novel object recognition was impaired in GSK3β, not GSK3α, knockin mice, but temporal order memory was not impaired in GSK3α or GSK3β knockin mice and coordinate spatial processing was impaired in both GSK3α and GSK3β knockin mice. Adult hippocampal neural precursor cell proliferation is impaired in GSK3α/β knockin mice and there was a severe impairment in GSK3β knockin mice, but no impairment in GSK3α knockin mice. These results demonstrate that hyperactivity of the two GSK3 isoforms display very different effects in the regulation of these processes. Specifically, increased activity of GSK3β, in the absence of additional disease pathology, is sufficient to impede mood regulation, novel object recognition, and hippocampal neural precursor cell proliferation, whereas hyperactive GSK3α does not impair these processes.

Disclosures: **M. Pardo:** None. **E. Abrial:** None. **R. Jope:** None. **E. Beurel:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

Location: Hall A

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Program#/Poster#: 727.01/BB51

Topic: F.02. Animal Cognition and Behavior

Support: JSPS KAKENHI 15H04265

Title: Synaptic plasticity with dendritic computing achieves the association of preplay patterns and place fields in hippocampus

Authors: *T. HAGA, T. FUKAI;
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Abstract: The firing sequence of hippocampal place cells experienced in exploration is repeatedly replayed in spontaneous activity. It is often bidirectionally replayed (forward replay and backward replay) and thought to be related to path integration and memory consolidation. However, it was recently found that the same sequence is observed before the experience, which is called preplay. This cannot be realized without the association of predefined firing sequences in the hippocampal recurrent circuit and novel sensory input patterns in the path that have never been experienced and it have not been realized in previous hippocampal models. In this presentation, we propose that such learning can be achieved by synaptic plasticity caused by dendritic calcium spikes of hippocampal pyramidal neurons. Calcium spikes in the apical dendritic shaft detect the coincident inputs between apical and basal dendrites (Shai et al., PLOS Computational Biology, 2015) and cause long-term potentiation of synaptic conductance (Gambino et al., Nature, 2014; Cichon et al., Nature, 2015). We assumed two-compartmental neuron model, and synapses in each compartment were modified by single-compartmental Bienenstock-Cooper-Munro (BCM) theory and a positive cross term that corresponds to the effect of calcium spikes. Using this model, we simulated CA3 network which has afferent inputs with random initial weights in apical dendrites and recurrent connections that spontaneously generate replays and theta sequences (Romani et al., Hippocampus, 2014) in basal dendrites. It could be mathematically proven that this learning rule maximizes the correlation of activations of two compartments. Furthermore, simulation results showed that, after learning, weight distribution of afferent synapses became spatially localized and neurons which were connected with strong recurrent connection acquired the similar receptive fields for adjacent positions. It means that place fields were created in association with preplay patterns. From these results, we concluded that synaptic plasticity caused by dendritic calcium spikes is one of considerable elements to model preplay.

Disclosures: **T. Haga:** None. **T. Fukai:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

Support: Israel Science Foundation Grant No. 1733/13

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Title: An efficient coding theory for a dynamic trajectory predicts non-uniform allocation of grid cells to modules in the entorhinal cortex

Authors: ***N. WEISS**[1], A. MORIEL[1], H. AGMON[2], Y. BURAK[2,1];
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Abstract: Recent experiments established that grid cells in the entorhinal cortex are functionally organized in discrete modules with uniform grid spacing. The ratios of grid spacing approximately form a geometric series. This result is in agreement with recent theories, which postulate that grid cells implement an efficient code for the animal's position. However, the experimental data suggests also that the number of cells decreases sharply with grid spacing, in marked disagreement with existing theories. Here, we postulate that the entorhinal cortex is adapted to represent a dynamic quantity (the trajectory of the animal in space), while taking into account the temporal statistics of this variable. We first develop a theory for efficient coding of a variable that dynamically follows the statistics of a simple random walk. A central prediction of the theory is that module neuron population sizes should sharply decrease with the increase of grid spacing, in agreement with the trends seen in the experimental data. Another prediction is that the ratio between grid spacings should approach a constant value in the modules with the smallest spacing. The predicted ratio is , which is consistent with experimental data and with previously proposed models. Next, we identify a remarkably simple, near optimal scheme for readout of the grid cell code by neural circuitry, in which model place cells linearly sum inputs from grid cells, using an exponential temporal kernel, whose decay time depends on the spacing of the presynaptic grid cell. Using estimates for the total number of grid cells in the entorhinal cortex, and the statistics of rodent motion, the time scales should range from ~1ms to ~1s, depending on the grid cell module. Thus, the simple readout requires mechanisms for persistence over this range of time scales. The simple readout scheme can be optimized for trajectories that deviate in their temporal statistics from a simple random walk. As an extreme case we consider motion at constant velocity in an unknown direction. Even for such motion, we obtain from the optimization qualitatively similar results as for random walk statistics. Thus, we propose that the

sharp decrease in module population sizes, with increase of the grid spacing, is an outcome of the efficient coding hypothesis, if the dynamic nature of motion in space is taken into account.

Disclosures: **N. Weiss:** None. **A. Moriel:** None. **H. Agmon:** None. **Y. Burak:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

Support: NSF/ANR Collaborative Research in Computational Neuroscience Grant: Spaquence

Title: Prefrontal cortex reservoir network learns to reconstruct navigation sequences by concatenating place-cell snippets replayed in hippocampus

Authors: N. CAZIN[1], J.-M. FELLOUS[3], A. WEITZENFELD[4], *P. F. DOMINEY[2];
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Abstract: Introduction: Spatial navigation in the rat involves the reactivation of hippocampal (HIP) place cells during quiet awake and sleep states. This generates short sequences of place cell activation in the order they are traversed that we call ‘snippets’. In a spatial learning task, the complete trajectory can be characterized by a set of overlapping snippets. We hypothesize that during active learning, the forward HIP replay of snippets contributes to the learning of the complete sequence in PFC. Methods: We model PFC as a recurrent reservoir network of 1000 leaky integrator neurons, with fixed recurrent inhibitory & excitatory connections, and modifiable readout connections. Readout neurons feed-back into the reservoir, allowing autonomous sequence reproduction and generation. We performed experiments where the model learned and generated the learned sequences autonomously, varying sequence length, complexity, reservoir size and noise. We then performed snippet experiments: complete sequences were split into partially overlapping subsequences of place cell activation. During training, snippets were presented in a random order. The system was then tested on its ability to generate the complete sequence after priming on the first 3 elements. Sequence length varied from 9 to 18, snippet length varied from 5 to 10, overlap varied from 2 to 8. We also tested a sparse snippet condition where elements were systematically eliminated from the snippets, so that no complete snippet was presented. Results: The reservoir displayed robust learning of

sequences of different length (10-20 elements) and complexity (1st and 2nd order). The reservoir displayed robust snippet learning, including reproducing a sequence of 18 elements, made from 3 snippets of length 10 and overlap 6. The sparse condition required 2000 neurons. Analysis of reservoir state trajectories by PCA demonstrates that the system exploits snippet overlap to learn the transitions between snippets and reconstruct the entire sequence. Discussion: These results are consistent with the hypothesis that snippet replay in HIP contribute to sequence learning in PFC. Snippets do not have to be contiguous, but they should maintain the correct order of place cell firing. This ability to align snippets depends on the fading memory property of the reservoir. This research demonstrates that the reservoir computing framework can provide novel insights into distributed coding in PFC.

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Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH MH083809

Title: Retrosplenial cortical neural populations simulate future trajectories

Authors: ***A. M. MILLER**[1], W. MAU[2], H. LI[1], K. YU[1], S. PARAUDA[3], D. M. SMITH[1]; [1]Cornell Univ., Ithaca, NY; [2]Boston Univ., Boston, MA; [3]Weill Cornell Med. Col., New York, NY

Abstract: The retrosplenial cortex (RSC) plays a prominent role in learning and memory. The RSC is a major component of the default network, a group of cortical memory structures involved in generative memory processes including autobiographical memory, constructive memory, and self-projection. Many of the structures that make up the default network have been implicated in the representation of future goals, outcomes, or behaviors. For example, in rats, the hippocampus has been shown to uniquely encode trial types associated with distinct trajectories (Wood et al., Neuron, 2000) and to represent spatial locations ahead of the rat during periods of vicarious trial-and-error (Johnson & Redish, J Neurosci, 2007). Similarly, we have found that many RSC neurons fire differently on the stem of a t-maze when during left and right turn trials in an alternation task (Miller et al, 2014, SFN Abs, 465.11). Given that fMRI findings suggest a role for the RSC in self-projection and generative memory processes, we hypothesized that

neural activity related to future goals and trajectories would be observable in rodents. Specifically, we test the hypothesis that the RSC encodes future spatial and reward locations by recording neural ensembles in the RSC of rats while they performed a continuous spatial alternation task that we have shown to require the RSC (Miller & Smith, 2012, SFN Abs, 706.06). Prior to training, a microdrive with 16 independently movable tetrodes was implanted bilaterally over the granular b region of the RSC. Recording began on the first day of training and continued through asymptotic performance. Consistent with previous results, we found that 46% of RSC neurons encoded spatial locations on the maze (i.e., place cells) and 58% encoded reward-location conjunctions. Additionally, we used Bayesian decoding to compute the rat's instantaneous location given ensemble spiking and compared the probability that the rat was located along its future trajectory, p(future), to the probability that the rat was located along the trajectory that it would not visit on that trial, p(non-visited). This analysis revealed that RSC neuronal populations more strongly represented future trajectories (p(future) - p(non-visited) was greater than 99.9% of shuffled outcomes). These findings provide a link between human literature on self-projection within the default network and neuronal recordings in rats during spatial navigation tasks, and are consistent with a role for the RSC in the use of memory representations to support the simulation of future behavior.

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Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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Support: DOD-ONR 26-1302-87xx

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CRSNS 26-1004-04xx

Title: An attractor model of probabilistic localization

Authors: ***I. KANITSCHEIDER**[1], A. POUGET[2,3,4], I. FIETE[5];
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Rochester, NY; [4]Gatsby Computat. Neurosci. Unit, London, United Kingdom; [5]Dept. of Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: To self-localize during navigation, animals integrate a wide range of spatial and movement cues of varying reliability. The optimal strategy would exploit all these cues, but with appropriate weighting. Reliability-unaware strategies are typically inaccurate and error-prone. How the brain computes and represents reliability, and how it uses this information in its position estimates is still largely unknown. In our model, an animal runs through a familiar one-dimensional track with a few landmarks. It has access to a noisy velocity signal (the noise is Gaussian) and when it encounters a particular landmark, it knows where it is up to some uncertainty described by a Gaussian of a known width for that landmark. In this scenario, a Kalman filter is the optimal location estimator. We search for a neural implementation of the Kalman filter with localized location tuning and coding of reliability. The first possibility is to encode location with a bump attractor, with bump height encoding the reliability of the estimate. For Gaussian tuning curves, the stable manifold of such an attractor is nonlinear, and we require a proper nonlinearity to project out deviations from the stable manifold. We show that an attractor with a divisive normalization-type nonlinearity can support Gaussian tuning curves of variable height. We then generalize the model so that it can perform location updates in response to a velocity signal, correctly decrement reliability in the absence of landmarks, and integrate landmark information when they are present. The experimental signature of such a model would be to find place or grid cells with lower firing rates when the reliability of location cues is reduced. The second possibility is to encode location with a one-dimensional attractor with fixed bump height and compute reliability in a separate population. The velocity signal updates the line attractor in a conventional manner, while the reliability information is used to calibrate the relative gain of the landmark input before it is used to update the bump position. Experimentally, this model predicts neurons purely tuned to reliability, but not to location. We comment on the advantages and disadvantages of each model and show that both perform very closely to the optimal Kalman filter. We also consider extensions of the model to the multidimensional case.

Disclosures: I. Kanitscheider: None. **A. Pouget:** None. **I. Fiete:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

Support: MURI Grant 201952

Title: A model of cognitive navigation inspired by the hippocampus

Authors: *A. V. SAMSONOVICH, G. A. ASCOLI;
Krasnow Inst. Adv Study, George Mason Univ., Fairfax, VA

Abstract: I am lost in a large, empty office building after business hours, and am looking for a restroom. As I enter a corridor from the staircase, I see an exit from the building on my right and an elevator on my left. I am intuitively confident I should turn left. As I pass the elevator, I spot a drinking water fountain on the right and a meeting room on the left. I head for the fountain and immediately see a restroom next to it. In contrast, the choices would be non-trivial for a state-of-the-art artificial intelligence (AI) system placed in the same situation. Indeed, efficient search strategies require either some prior knowledge of the environment (which is not assumed) or some human-level general world knowledge; otherwise uninformed search would be deemed the best strategy. Most spatial learning and navigation problems today are not considered AI-hard. Yet, there is a certain class of seemingly simple problems that still resist mainstream approaches. We call them cognitive navigation problems, referring to navigation of unknown or unpredictable environments that may require analogical reasoning, generalization, common sense, intuition, creativity, insight, theory of mind, and other forms of imprecise inductive reasoning. AI typically attempts to implement these functions using top-down approaches. Yet, we usually solve such problems in real life without logical reasoning. This work explores a plausible account of this process based on statistical learning of remotely similar environments in a model inspired by our previous studies of the rodent hippocampus (Samsonovich and Ascoli, Learning & Memory 2005; BICA 2013). A test paradigm to compare model and human performance involves exploration and then navigation of previously unseen, randomly generated labeled graphs. Features that are close to each other in one graph are typically found close to each other (if present) in other graphs as well; otherwise the graphs are dissimilar. The model network includes three layers: place cells, feature cells, and goal cells. Neuronal activity represents the current location of the actor. Exploration of available moves reveals nearby features that serve as cues in pathfinding. Preliminary simulations using graphs of 100 nodes with 30 kinds of features show that the network reaches the goal in an unseen graph after 15.1 steps on average, using less than 100 steps in 96% of all sessions. The resultant solution outperforms traditional methods, including Q-learning and Canadian Traveller Problem solvers. Expected outcomes include big data search tools, personal assistants, and a new insight into the hippocampal function. We are grateful to Prof. Theodore C. Dumas for valuable discussions.

Disclosures: A.V. Samsonovich: None. **G.A. Ascoli:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 727.07/BB57

Topic: F.02. Animal Cognition and Behavior

Title: A neural computation model of the goal direction based on the reactivation of place cells, grid cells and stripe cells

Authors: *J. K.-S. LAI[1], M. S. MITSUZAWA[2], Y. YAMAGUCHI[2];
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Abstract: Place cells in the hippocampus and grid cells in the entorhinal cortex are known to be key components of the navigation system in rodents and primates. In addition, grid cells are known to show band-like firing properties. These cells may reflect neurons with a spatially periodic firing pattern with a conjunctive head direction preference as a subclass of grid cells, what we call stripe cells below. Although various types of neural firing have been experimentally demonstrated, the neural network mechanism for spatial navigation is still an open question. In this paper, we propose a model of neural computation of goal direction from a given position. The model consists of a stripe cell layer, a grid cell layer, a place cell layer and the reactivation monitoring system. The stripe cells have the ability of displacement integration with head direction preference, a group of which can generate the firing of grid cells. Each place cell has an association with a population of grid cells. We assume that the reactivation of place cells in a random manner is associated with stripe cells and grid cells. The reactivation monitoring system is composed of circular neural network attractors with input from stripe cells. The random displacement in arbitrary reactivation can be integrated to give a population vector representing the shortcut pathway to the goal. Our computer simulation successfully demonstrated the computation of a goal direction population vector in the reactivation monitoring system using continuous attractors. Thus, our model suggests the importance of concurrent reactivations of place cells and grid/stripe cells in navigation.

Disclosures: J.K. Lai: None. **M.S. Mitsuzawa:** None. **Y. Yamaguchi:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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National Institute of Mental Health Grant RO1 MH079971

Title: Distal cue configuration-dependent rate remapping in the hippocampal place cells in an allocentric memory task

Authors: *S.-B. PARK, I. LEE;
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Abstract: Rats use distal cues for allocentric spatial navigation and the firing patterns of hippocampal place cells are influenced by distal cues. However, distal cue manipulations were made irrespective of task demand in most studies, or no memory demand was present between particular distal cue configuration and behavior. We investigated whether place cells fired in association with specific distal cue configurations in a task-dependent manner if a behavioral task required so. Rats (n=4) ran along a linear track at the end of which a choice should be made between two food wells. A correct response was associated with a particular distal cue configuration. A distal cue configuration was defined by the angular distance between two curtains (cue curtains) each of which contained a set of visual cues. The cue curtains were movable along the ceiling curtain track to make the following angular distances between the curtains: 0°, 14°, 66°, and 80°. The rat was initially trained to choose the left food well when the cue curtains were separated by 0°, but the right food well for 80°. After the rat learned the task to criterion, a hyperdrive was implanted and the rat was overtrained with the same paradigm. Once the recording began, the new cue configurations (14° and 66°) were intermixed with the original configurations. When facing the new configuration, the rat was required to choose the left or right food well according to its angular proximity to the original cue configurations. Rats were able to make correct choices significantly at above chance for all cue configurations. Our previous study demonstrated that inactivating the dorsal hippocampus impaired normal performance in this task. We recorded putative complex spiking units (n=114) simultaneously in CA1 of the dorsal hippocampus as the rat performed the task. There was minimal spatial remapping in place fields across different cue configurations within a session, but diverse patterns of in-field rate modulation (rate remapping) were observed in place cells across cue configurations as follows: (i) upcoming response-related firing (elevated firing for a particular food-well choice), (ii) cue novelty-related firing (elevated firing for either original or newly

introduced cue configurations), and (iii) mixed firing (rate modulation patterns not belonging to either (i) or (ii)). The three types of cells seem to exist at equal proportions in the hippocampus (approximately 33% for each). We will further investigate this neural phenomenon to find whether these neuronal firing patterns can predict the rat's behavior in the task.

Disclosures: S. Park: None. **I. Lee:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

Support: NRF Grant 2013-R1A1A2053280

Title: Computational investigation of the direct transformation of grid cell spike activities into hippocampal ramp-like input and spike phase precession of place cell

Authors: *S. PARK, J. KWAG;
Dept. of Brain and Cognitive Engin., Korea Univ., Seoul, Korea, Republic of

Abstract: Hippocampal place cell is a spatial information processing neuron where individual spike of place cell within the place field shows spike phase precession; spike phases advance 360° relative to the hippocampal theta oscillation, coding for the specific location within the place field. However, the exact mechanism underlying the spike phase precession is still unclear. Whole-cell patch-clamp recording of place cells in the hippocampal CA1 *in vivo* revealed that place cells receive asymmetric excitatory ramp-like input (ERI) in the place field. Therefore, investigating the source of hippocampal ERI could shed light on the cellular and network mechanism underlying spike phase precession. Since neurons in hippocampal CA1 receive excitatory afferent directly from layer III of medial entorhinal cortex (MEC), it is possible that the grid cells in MEC, another spatial information processing cells that spike in grid-pattern, could contribute to the ERIs observed in place cells and consequently to phase precession. Therefore, we used the oscillatory interference (OI) grid cell model and Hodgkin-Huxley (HH) place cell model to investigate the above hypothesis. Simulated grid cell spikes were modeled to give excitatory synaptic input to a distal dendrite of the HH place cell model using the single exponential function to mimic MEC-CA1 connection. Simulations and analysis were done using the NEURON and MATLAB. Each simulated grid cell spike was transformed into excitatory postsynaptic potentials (EPSPs) in the hippocampal place cell HH model, and grid-patterned

spikes resulted in summated EPSPs, giving rise to ERIs. 45% of transformed ERI were right-skewed (R-ERI), 22% were symmetric ERI (S-ERI), and 33% were left-skewed ERI (L-ERI). When R-ERI was superimposed with theta oscillation in the place cell HH model, spike phase precession occurred over distance with strong negative correlation ($\rho = -0.58$). In contrast, L-ERI showed spike phase recession with positive correlation ($\rho = 0.68$), and S-ERI had nearly no correlation ($\rho = 0.09$). Our simulation results show for the first time that grid cell spikes providing excitatory input to the place cell can be directly transformed into different shapes of hippocampal ERIs. Moreover, R-ERI with theta oscillation could give rise to spike phase precession while L-ERI could give rise to spike phase recession. Direct transformation of grid cell spikes into hippocampal phase precession suggests that grid cell activity could serve as one mechanism underlying place cell spike phase precession. Future *in vivo* testing of our results will lead us to better understand spatial information processing in the entorhino-hippocampal circuitry.

Disclosures: S. Park: None. **J. Kwag:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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BMBF 01GQ1004A

Title: Why the entorhinal grid map is discretized: how a geometric progression of grid scales enables goal-directed navigation, error correction and modular arithmetic

Authors: *M. B. STEMMLER[1], A. MATHIS[2], A. V. M. HERZ[1];
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Abstract: Grid cells in medial entorhinal cortex exhibit a discrete set of length scales, such that the representation of space ranges from coarse to a fine (Stensola et al. 2012). Discrete modules permit a biologically plausible population vector read-out of the distance and direction towards goal locations (Stemmler et al. 2014). We show that such a read-out can be made robust to distortions in the regular grid pattern near the boundaries of the environment (Stensola et al.

2015) to permit a global grid-cell metric. While a continuum of grid scales would also permit a population-vector read-out, we prove that significant precision is lost in a continuum, even though an ideal observer receives the same amount of spatial information from the population of grid cells. The maximal spatial range represented in the grid code is the product of all scales, provided that no two scales can be evenly divided by the same (non-trivial) integer factor (Fiete et al, 2008). Such an arrangement would permit the grid code to function as a *residue number system.* We quantify the likelihood of making catastrophic mistakes in a probabilistic model of grid cell firing. In the limit of high noise and low firing rates, we show that the grid code should not use a classical residue number system. Instead, the optimal grid code relies on a geometric progression of scales so that the ratio from one scale to the next is fixed to 3:2. This strategy reduces the spatial range encoded in the population to 2^{L-1} times the longest length scale, where L is the number of discrete modules, but minimizes mistakes.

Disclosures: M.B. Stemmler: None. **A. Mathis:** None. **A.V.M. Herz:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

Location: Hall A

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Program#/Poster#: 727.11/BB61

Topic: F.02. Animal Cognition and Behavior

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Stiftung Mercator

Title: From grid cells to place cells with realistic field sizes

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Abstract: Grid cells in the medial entorhinal cortex (EC) have spatial receptive firing fields (place fields) highly ordered on a hexagonal grid. However, just one synapse downstream, hippocampal place cells mostly express one large place field. An ongoing debate in the field is about whether the place cells are created by the input from grid cells in a simple feedforward network. Initially, after the discovery of grid cells, it was widely believed that the simple

transformation form grid to place cells was plausible. However, recent experiments have called this hypothesis into question, when they found that grid cells were highly degraded in certain conditions, while, at the same time, place cells appeared normal. Then again, subsequent modeling studies have found evidence that these experimental results are consistent with a transformation form grid to place cells in a feed forward network since the transformation is robust to noise in the grid cell inputs. However, all the extant models that produce place cells with robust place fields suffer from an issue that has received little attention so far. The place fields are rather small, not comparable to the place fields found in the CA regions. Here, we first study the computational issues for the creation of large single fields solely out of grid input in such a network. We then propose a model that generates place cell firing based on inputs from grid cells and nonspatial cells, that are only weakly spatially modulated and abundant throughout the EC. This simple model reproduces many place cell characteristics as well as results from studies in lesioned animals and make some clear predictions. This work strongly suggests that place cells are not the result of feedforward drive from grid cells alone.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: DFG, SFB 874 B2

Title: A computational model of information-flow in the hippocampal formation of the rat

Authors: ***M. PYKA**[1], S. CHENG[2];
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Abstract: The hippocampus is a subcortical structure that is not only known for its important role in episodic memory and navigation but also for its complex 3-dimensional shape. Within and across species, there are differences in its absolute size, its exact shape and its embedding in the rest of the brain (Manns & Eichenbaum, 2006)□. Its internal structure and function are, however, assumed to be conserved across mammals (Allen & Fortin, 2013; Manns & Eichenbaum, 2006)□. Since changes of the form, size and connectivity of network structures are very prominent traits of evolutionary variations, one interesting question is: Does the morphology of the network have an affect on its functioning? To investigate this question, we

created a 3d-anatomical model of the rat hippocampus using Parametric Anatomical Modeling (PAM). PAM facilitates the translation of large-scale anatomical data into a formal description of neural networks with connection patterns and connection lengths derived from anatomical features of the biological network (Pyka and Cheng 2014). Based on this model, we computed connection lengths between entorhinal cortex and various areas within the hippocampal loop. In combination with axon caliber distributions reported by experimental studies transmission delays between the subregions in the hippocampal formation were calculated. With these data, we reconstructed the information flow within the hippocampal loop, that is the timing and temporal order, in which spiking activity propagates through the network. Our simulation results are consistent with reports from experimental studies (Mizuseki et al. 2009, Montomgery et al. 2009) and predict delay-distributions that can be experimentally tested in future studies. Moreover, we found in some areas (e.g. entorhinal cortex, CA3 and CA1) significant dependencies between transmission time and position of the neuron along anatomical axes. Thereby, the data provide estimates of how spatial relations between different brain areas affect functional and other structural properties of the network. Additionally, our simulations of information flow constrain the space of computational models of hippocampal functions.

Disclosures: **M. Pyka:** None. **S. Cheng:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

Title: A parametric anatomic model of the pigeon hippocampal formation

Authors: *R. GÖRLER[1], O. GÜNTÜRKÜN[2], M. PYKA[3];
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Abstract: Comparative analyses of brains of different species are indispensable in order to understand how function emerges from structure. One key idea is that fundamental properties of neural circuits and information flows are broadly conserved across species despite tremendous species-specific differences in size, shape and connection scheme of the network. The hippocampus is a prime example suitable for studying general principles of nervous system organization as it seems to be structured differently in mammals and birds but is known to play an important role in memory consolidation and spatial navigation in both taxa. The pigeon

(Columba Livia) with its extraordinary navigational abilities is, therefore, a promising candidate for comparative studies of the hippocampal formation. In this study, we present a three-dimensional model of the pigeon hippocampal formation that was constructed using Parametric Anatomical Modeling. This modeling technique facilitates the translation of large-scale anatomical data into a formal description of neural networks with connection patterns and connection lengths derived from anatomical features of the real network. The pigeon hippocampus model comprises the state of research regarding subdivisions and interconnections while allowing for the connection properties to be further parametrized. We demonstrate how the model can be used to reveal temporal patterns of network activity based on connection lengths computed from the 3d model and axon diameter distributions. We further provide several estimates about the information flow in the pigeon hippocampus which can experimentally be tested in future studies. In combination with a previously presented 3d hippocampus model of the rat, we show how 3d computational models can be exploited to help comparative neuroscience to identify common and species-specific features of the hippocampal formation.

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Poster

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Support: Stiftung Mercator

SFB 874 project B2

Title: The role of semantic representation in episodic memory

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Abstract: It is widely, albeit not universally, believed that episodic and semantic memory are distinct forms of memory. Mounting experimental evidence suggests that the hippocampus is required for episodic memory, but not for semantic memory. The latter appears to be stored solely in the neocortex. This dissociation does not, however, imply that episodic and semantic memory operate independently or recruit non-overlapping brain resources. Over the years, many authors have suggested various theories about the interdependence of episodic and semantic

memory. Tulving's SPI model (1995) specifies different kinds of interdependence: serial encoding, parallel storage and independent retrieval. While Baddeley (1988) viewed semantic memory as the "accumulated residue" of multiple learned episodes. Recently, Greenberg and Verfaellie (2010) suggested that semantic knowledge provides a framework or scaffolding which facilitates the acquisition of new episodic memory. Here we propose a computational model of the interrelation between episodic and semantic memory. We hypothesize that episodic memories are represented as sequences of activation patterns that are stored in the hippocampus. These patterns are the output of a semantic representational network, in the neocortex, that compresses the high-dimensional sensory input to a lower dimensionality both in space and time. That is the semantic representation can be represented by fewer neurons and varies at a lower rate than the sensory input stream. Since the hippocampal patterns are highly compressed inputs, our model accounts for findings that episodic memory is unreliable in humans, often preserving little more than the gist of the episode, but none of the details. Using this computational model, we show that the accuracy of episodic memory critically depends on the semantic representations by comparing retrieval accuracy in two conditions. In one case, episodic memories are stored using a semantic representation that was trained on the same input statistics (appropriate representation). In the other case, the semantic representation was trained on a different kind of input statistics (inappropriate representation). Retrieval using the appropriate representation was superior to that based on the inappropriate representation. We confirm this modeling result in a behavioural experiment. We thus conclude that semantic representation plays an important role in episodic memory.

Disclosures: **J. Fang:** None. **S. Cheng:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

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R01MH090188

Title: A hippocampal network for spatial coding during immobility

Authors: *K. KAY[1], M. SOSA[1], J. CHUNG[1], M. P. KARLSSON[1], M. C. LARKIN[2], I. GROSSRUBATSCHER[1], L. M. FRANK[1];
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Abstract: Classic hippocampal "place" cells are active in discrete locations as subjects traverse space. This place activity constitutes an explicit and precise neural code for location in the mammalian brain. Indeed the hippocampus is required for memory-guided spatial navigation, a behavior understood to be dependent on an internal representation of current position. However, studies of hippocampal place firing conventionally exclude periods of immobility. As a result, the fundamental issue of whether and how the hippocampus represents current position in the absence of movement has stood unresolved. In rats engaged in a spatial memory task, we recently found a population of neurons, located in hippocampal area CA2, that fired at specific (reward site) locations during periods of immobility. Spike-triggered analysis of LFP subsequently showed that these neurons associate with a non-canonical hippocampal network pattern (NCP): a ~200 ms, low frequency positive "wave" pattern detectable in CA3 and DG. Here we report that putative interneurons located throughout the hippocampus associate with the NCP, and further that CA1 and CA3 principal neurons associating with the NCP also show profound location specificity during immobility. These results identify a hippocampal network pattern, distinct from both hippocampal theta and sharp-wave ripples, that operates during immobility. Moreover the association of this pattern with location-specific firing indicates that the hippocampus, during immobility, is actively engaged in generating a representation of current position. Our results also demonstrate rapid switching in the hippocampal circuit between representation of current position and SWR-associated representations of past (“replay”) and potential (“preplay”) navigational experience.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: SFB Grant SFB874; project B2,B3

Title: Self-organized formation of place cell responses in robotic simulations based on slowness principle

Authors: *S. KUMAR[1,2], F. SCHÖNFELD[2,3], L. WISKOTT[2,3], S. CHENG[1,2];
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Abstract: Since the discovery of place cells four decades ago, several studies have been conducted to investigate the properties of and inputs to the place cells. However, it remains unclear what information drives place cell responses and how the brain might extract such information. Franzius et al1 previously suggested that an unsupervised learning algorithm could learn place, head direction and spatial view cell responses solely based on visual inputs. This algorithm uses a hierarchical network of slow feature analysis (SFA) nodes and exploits the fact that the pixels in the visual input vary much faster than the underlying spatial parameters that determine the visual input. However, that study simulated a virtual environment under idealized conditions with the behavior of the animal perfectly controlled. Such conditions are not representative of the real world. Here, we apply the SFA-based algorithm on data collected in robotics experiments using the small e-Puck robot and a virtual e-Puck simulated in Webots. These experiments are constrained by physical variables such as noise and distortions in the camera, lighting conditions, inertia, friction, and limits on speed and acceleration. We have used the slowness principle to explain some of the distinct properties of place cells observed in studies such as direction dependence on the linear track or direction invariance in open arena. Preliminary analysis of these responses in a linear track and an open arena suggest that spatially selective responses also arise in robotics simulations, although it remains a challenge to clearly separate responses to location, orientation and view. We tentatively suggest that the SFA-based algorithm is robust to the noise and limits imposed by physical constraints, which animals also face in the real world. [1] Franzius, Mathias, Henning Sprekeler, and Laurenz Wiskott. "Slowness and sparseness lead to place, head-direction, and spatial-view cells." PLoS Computational Biology 3.8 (2007): e166..

Disclosures: **S. Kumar:** None. **F. Schönfeld:** None. **L. Wiskott:** None. **S. Cheng:** None.

Poster

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Title: Apolipoprotein E4 impairs slow gamma oscillations during hippocampal sharp-wave ripples in a mouse model of Alzheimer's disease

Authors: *A. GILLESPIE[1], Y.-H. LIN[1], M. KARLSSON[2], K. KAY[2], S. YOON[1], L. FRANK[2], Y. HUANG[1];
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Abstract: Apolipoprotein (apo) E4 is the major genetic risk factor for Alzheimer's disease (AD), yet we do not understand how it alters memory processes to ultimately cause cognitive decline. As the hippocampus is critical for spatial learning and memory, and is one of the brain regions first and most severely targeted by AD pathology in humans and disease models, we examined apoE4-induced changes in hippocampal network activity. In knock-in (KI) mice, human apoE4 causes age-related learning and memory impairment as well as GABAergic interneuron degeneration in the dentate gyrus (DG) of the hippocampus. *In vivo* local field potential recordings throughout the hippocampus of aged apoE4-KI mice revealed two alterations impacting sharp wave ripple (SWR) events, which are critical for memory processes. Compared to control apoE3-KI mice, apoE4-KI mice have fewer SWR events. Furthermore, we observe SWR-associated slow gamma activity in hippocampal subregions CA1, CA3, and DG, and demonstrate that this SWR-associated slow gamma power is significantly attenuated throughout the hippocampal circuit in apoE4-KI mice. Elimination of apoE4 in GABAergic interneurons, which prevents learning and memory impairment, rescued SWR-associated slow gamma activity but not SWR abundance. These results suggest that the disruption of DG-enabled slow gamma activity during SWRs is a key mechanism of apoE4-mediated learning and memory impairment.

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Poster

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Title: Real-time estimation of hippocampal replay content

Authors: *X. DENG[1], D. F. LIU[2], M. KARLSSON[3], L. M. FRANK[3], U. T. EDEN[1];
[1]Mathematics and Statistics, Boston Univ., Boston, MA; [2]UC Berkeley - UCSF Grad. Program in Bioengineering, [3]Physiol., UCSF, San Francisco, CA

Abstract: Sharp-wave ripple (SWR) events in the hippocampus replay millisecond-timescale patterns of place cell activity related to the past experience of an animal. Previous work has shown that interruption of awake SWRs leads to a specific learning and performance deficit. That result established the importance of awake SWRs, but as individual SWRs can contain spiking patterns that replay different sequences, the role of specific sequences in learning and decision-making is not known. A deeper understanding of how this replayed information contributes to learning and decision-making will require the ability to manipulate SWR events based on their content. Here our goal is to develop a decoding algorithm to determine if a replay event's content represents a specific sequence, which will then make it possible to interrupt events based on the spatial trajectory they represent. Accurate real-time decoding of SWR replay content requires new algorithms that estimate trajectory and the associated uncertainty, along with software and hardware that can execute these algorithms for biological interventions on a millisecond timescale. Previously, we developed an efficient decoding algorithm that does not require multiunit spiking waveforms to be sorted into single units (Deng et al., 2015). Here we extend the state of our marked point process filter to include a dynamic binary state variable that categorizes the content of hippocampal replays in real time. This allows us to incorporate into our model three fundamental features of the information content of individual replay events: the location where the trajectory begins, whether the sequence of locations represents a trajectory proceeding toward or away from the animal's current position, and whether the spiking pattern reflects place-field structure for a specific direction of movement. Using Bayes' rule, we compute the posterior distribution of the binary state variable to determine the confidence that the replay content represented in hippocampal multiunit activity represents inbound versus outbound trajectories. We illustrate our approach by decoding experimental data recorded in the hippocampus of a rat performing a spatial memory task. This algorithm is suitable for a real-time implementation with short latencies to incorporate into content-based feedback experiments.
Deng, X., Liu, D.F., Kay, K., Frank, L.M., and Eden, U.T. (2015) Clusterless Decoding of Position From Multiunit Activity Using A Marked Point Process Filter. Neural Computation.

Disclosures: **X. Deng:** None. **D.F. Liu:** None. **M. Karlsson:** None. **L.M. Frank:** None. **U.T. Eden:** None.

Poster

727. Cortical and Hippocampal Circuits: Models of Spatial Navigation

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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano.

Title: Data-driven hippocampus ca1 modeling in the human brain project

Authors: ***A. ROMANI**[1], N. ANTILLE[1], J. A. DYNES[1], J. FALCK[2], M. GEVAERT[1], L. KANARI[1], J. G. KING[1], S. LANGE[2], A. MERCER[2], E. B. MULLER[1], S. RAMASWAMY[1], M. W. REIMANN[1], L. R. J. RIQUELME[1], C. A. RÖSSERT[1], Y. SHI[1], M. TELEFONT[1], A. THOMSON[2], W. A. H. VAN GEIT[1], H. MARKRAM[1];
[1]Campus Biotech, EPFL ENT CBS BBP, Geneva, Switzerland; [2]UCL, London, United Kingdom

Abstract: Given its importance in many brain functions, and due to the richness of the data available, the hippocampus was selected as one of the first brain regions to be modeled by the Human Brain Project (HBP). The HBP approach to building computational models of brain regions offers a powerful tool to integrate the available data and predict the unknowns. The available information for a given brain region, even for the most well-studied regions such as the hippocampus, is highly sparse and incomplete, and we are far from filling all the gaps. Nevertheless, methods have been developed within the HBP that start from existing knowledge, and algorithmically predict the missing data. We present a first draft model of the hippocampus

CA1 microcircuit of an adult rat. The microcircuit is a compound of a series of component models for ion channels, neuron anatomy and physiology, synapse anatomy and physiology, as well as circuit anatomy and physiology. Once a component model is constrained and validated against the available experimental data, its parameters are frozen before the model is incorporated into the next level. This forces the modelers not to change the parameters ad hoc in order to reproduce a subset of experimental data or test a particular hypothesis, but allows them instead to remain in a more general and hypothesis-free framework. For each of the most well-characterized cell types, we collected one or more Neurolucida 3d digital reconstructions. We then positioned the morphologies inside the circuit according to information on localization and composition as outlined in the literature. A connectome was predicted on the basis of vicinity - if two morphologies are located in close vicinity inside the circuit, there is a higher likelihood of connection between them - and then scaled down to reproduce biological values of bouton density and synapses per connection. A number of electrophysiological recordings and published data were used to constrain models of single cell and synapse physiology. Finally, a dataset was used to validate the full circuit. The model represents a useful platform to integrate data, to test hypotheses and support experimentations, and it can be improved periodically by refining previous assumptions and incorporating new experimental data.

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Poster

728. Fear Memory: Molecular Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Title: Differential rearing effects on NR2B subunit expression in the rat amygdala and hippocampus during acquisition of Pavlovian conditioned fear

Authors: *L. E. KOMER[1], E. K. REINHARDT[2], G. R. ERICKSON[2], M. E. CAIN[2]; [1]Psychological Sci., [2]Kansas State Univ., Manhattan, KS

Abstract: Early rearing conditions may alter the development of anxiety disorders in individuals. Not only may the rearing conditions affect the expression of such disorders, but they

also may affect the initial learning of fear. In order to understand the influence of early rearing conditions on fear learning, an animal model of differential rearing is used (Renner & Rosenzweig, 1987). Rats raised in enriched conditions (EC) have superior learning and memory compared to standard condition (SC) and isolated condition (IC) rats (Wood and Rebec, 2009). However, IC rats appear to have superior learning of aversive stimuli (Walasek et al., 2002). Differential rearing also appears to affect the NR2B subunit of the glutamatergic N-methyl-D-aspartate (NMDA) receptor, which is involved with fear learning and memory (Rodrigues et al., 2001). The present study examines if NR2B differences among rearing environments are found in the amygdala, specifically the basolateral (BLA) or the central nucleus (ACe), and in the hippocampus, specifically the CA3 region. We hypothesized that IC and SC rats would acquire fear faster than EC rats. Similarly, we predicted that IC and SC rats would show more NR2B expression in the BLA, the ACe, and the CA3. Male Sprague-Dawley rats arrived at the laboratory at 21 days of age and were randomly assigned to the EC, SC, or IC, and reared for 30 days. All rats were trained to lever press for sucrose on a VI-90 schedule of reinforcement. Rats then received one 90-min fear acquisition session. Half of the rats received four presentations of a 3000-Hz tone (CS) paired with a 0.6-mA foot shock (US) and the other half of the rats received four unpaired presentations of the tone and foot shock. Fear was measured using a suppression ratio. Immediately following the fear acquisition session, the brains were extracted and the expression of NR2B was labeled and quantified in the BLA, ACe, and CA3 using immunocytochemistry. Results revealed only the IC paired rats acquired fear. In all 3 rearing conditions, no differences in NR2B expression were observed between the paired and unpaired rats, suggesting that the acquisition of fear did not alter NR2B expression. Interestingly, in the BLA, EC rats had significantly higher levels of NR2B expression than IC rats. No significant differences in NR2B expression were found in the ACe or CA3 regions. These findings suggest that while isolation rearing enhances the early acquisition of fear, this enhancement is not due to increased expression of NR2B receptors. These findings further suggest that enrichment increases NR2B expression within the BLA and suggests that the effect of enrichment on NR2B expression is regionally specific.

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Poster

728. Fear Memory: Molecular Mechanisms

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Title: NMDA receptor-dependent signaling in excitatory prefrontal neurons controls fear discrimination and fear extinction

Authors: *A. CORCHES, P. VIEIRA, N. BAVADIAN, A. HIROTO, K. WESTBROOK, E. KORZUS;
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Abstract: N-methyl-D-aspartate receptors (NMDARs) are implicated in synaptic plasticity and memory function including modulation of fear memory. NMDAR-dependent plasticity is widely hypothesized to be the mechanism by which memory traces are encoded and stored. Previous studies investigating fear memory focus on the amygdala and the hippocampus but the role of the medial prefrontal cortex (mPFC) in modulation of fear memory is less clear. Neurons in mPFC have been implicated in fear memory extinction. In addition, recent studies implicated prefrontal circuitry in fear memory specificity and generalization. We tested mice with locally deleted Grin1 gene (encoding the obligatory NR1 subunit of the NMDAR) from prefrontal CamKIIα positive neurons for their ability to distinguish aversive and harmless stimuli in a fear discrimination test. These mice showed impaired fear discrimination following initial generalization of conditioned fear. Mice with GRIN1 deletion in excitatory prefrontal neurons exhibit normal fear responses to aversive fear-conditioned stimuli but fail to reduce their fear response to non-aversive stimuli. These data provide evidence that NMDARs in the mPFC are part of a neural mechanism supporting discriminatory fear learning. We also found that NMDAR-dependent signaling in the mPFC is crucial for fear extinction of auditory conditioned stimuli. These studies suggest that the mPFC is required for a reduction of generalized fear to harmless stimuli that is essential for improvement of fear memory accuracy. These studies have clinical implications. Overgeneralized fear is a typical symptom of anxiety disorders including generalized anxiety disorder and posttraumatic stress disorder (PTSD), which are triggered by cues in a secure environment that resemble those of the traumatic experience.

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728. Fear Memory: Molecular Mechanisms

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Title: Trace-fear conditioning alters the expression of NMDA receptor related genes in relevant brain regions

Authors: *D. T. BALU, K. T. PRESTI, J. T. COYLE;
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Abstract: Fear conditioning is one of the most powerful and widely used models for elucidating the neural substrates of associative learning and memory formation in the mammalian brain. In this form of Pavlovian conditioning, a neutral stimulus (conditioned stimulus; CS) acquires predictive value by pairing it with an aversive, unconditioned stimulus (US; mild foot shock) that has an intrinsic value to the subject. After training, exposure of the animal to the CS or context alone elicits conditioned fear responses. The insertion of a trace interval during CS-US presentation alters the brain circuitry that mediates conditioning. There is substantial evidence that N-methyl-D-aspartate receptors (NMDARs) in the amygdala, hippocampus, and medial prefrontal cortex (mPFC) are involved in trace-fear conditioning. We previously demonstrated that D-serine, the co-agonist at forebrain NMDARs, is required for the development of contextual fear conditioning. Accordingly, we now examine whether the expression of genes related to NMDAR activity is altered after conditioning and re-exposure to the conditioning context. We used adult, male C7BL6 mice for all experiments. Mice were either: 1) naïve, 2) trace-fear conditioned and killed 60min after training, or 3) trace-fear conditioned, re-exposed to the training context the next day (no tones), and killed 30min later. We also included additional sham control groups that went through the same conditioning procedures, but did not receive foot shocks. We found that protein levels of the immediate early gene, activity-regulated cytoskeleton-associated protein (Arc; Arg3.1), were robustly increased after training and after context re-exposure. The animals exposed to sham conditioning showed a modest Arc protein increase only in the PFC after training, but no significant increase after context re-exposure. Notably, the mRNA and protein expression of serine racemase, the enzyme that converts L-serine to D-serine, was significantly increased in the amygdala after training and after re-exposure. SR protein did not change in sham mice at either time point. Using immunofluorescence, we found that SR and Arc are localized to distinct neuronal populations in

the amygdala after fear conditioning. These findings demonstrate an activity-dependent regulation of SR in the amygdala and confirm that D-serine is important for the acquisition and expression of fear memory.

Disclosures: **D.T. Balu:** None. **K.T. Presti:** None. **J.T. Coyle:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A patent owned by Massachusetts General Hospital for the use of D-serine as a treatment for serious mental illness could yield royalties for J.T.C. F. Consulting Fees (e.g., advisory boards); J.T.C. has served as a consultant for EnVivo, and Abbvie in the last 2 years..

Poster

728. Fear Memory: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 728.04/BB73

Topic: F.02. Animal Cognition and Behavior

Support: NIMH IRP

Title: Remote fear memory is regulated in the medial amygdala via tuberoinfundibular peptide of 39 residues acting through parathyroid hormone 2 receptors

Authors: *M. C. TSUDA, T. B. USDIN;
Section on Fundamental Neurosci., NIMH/NIH, Bethesda, MD

Abstract: Individuals with post-traumatic stress disorder have enhanced fear responses to trauma-associated stimuli, which is thought to result from impaired extinction of fearful memories. Our recent neuroanatomical and behavioral findings suggest that the neuropeptide tuberoinfundibular peptide of 39 residues (TIP39), via its receptor, the parathyroid hormone 2 receptor (PTH2R), modulates fear memory (Coutellier and Usdin, Behav Brain Res, 2011). We are now investigating the anatomical and cellular localization of TIP39's contribution to enhanced remote fear memory. PTH2R knockout (PTH2R-KO) and wild-type (WT) male mice were exposed to a single 2 second 1.5 mA foot shock and fear recall was assessed as conditioned freezing during re-exposure to the shock context 28 days later. Compared to WT, PTH2R-KO mice had increased freezing in the shock context, suggesting enhanced remote fear memory in mice that lack TIP39 signaling. The medial amygdala (MeA), which contains a high density of PTH2Rs and TIP39 containing terminals, projects to brain regions with well established roles in fear memory including other parts of the amygdala and the bed nucleus of the stria terminalis. We investigated the contribution of TIP39 signaling in the MeA to remote fear memory

enhancement by (1) blocking TIP39 signaling in the MeA or (2) inhibiting the activity of PTH2R expressing neurons in the MeA. TIP39 signaling in the MeA was blocked by stereotaxic injection of a virus encoding a secreted PTH2R antagonist (HYWH). Mice then received a foot shock as described above and fear recall was measured 28 days later. Similar to KO results, mice injected with HYWH had higher levels of freezing than controls. We then used the designer receptor exclusively activated by designer drug (DREADD) pharmacogenetic technique to ask whether signaling through cells that TIP39 acts on in the MeA is required during initial coding and/or recall of fear memory. A Cre-dependent Gi-coupled DREADD virus that can suppress neuronal activity was injected into the MeA of mice with PTH2R driven Cre expression. Saline or clozapine-N-oxide (CNO), a DREADD agonist that has no effect on endogenous receptors, was administered (1) 1 hour before foot shock or (2) 1 hour before fear recall testing. Fear recall was evaluated 28 days after foot shock. Inhibiting PTH2R expressing neurons with CNO at the time of foot shock increased freezing during fear recall, but inhibition at the time of recall had no effect, compared to saline treated mice. Taken together, these findings demonstrate that TIP39 signaling within the MeA at the time of an aversive event contributes to modulation of long-term fear recall of traumatic experience.

Disclosures: **M.C. Tsuda:** None. **T.B. Usdin:** None.

Poster

728. Fear Memory: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 728.05/BB74

Topic: F.02. Animal Cognition and Behavior

Title: Contextual fear-conditioning alters phosphodiesterase-4A intron expression in the hippocampus

Authors: *R. HANSEN, III[1], S. POPLAWSKI[2], G. PORCARI[1], R. HAVEKES[1], L. PEIXOTO[3], T. ABEL[1];
[1]Biol., Univ. of Pennsylvania, Philadelphia, PA; [2]Ibis Biosci., Carlsbad, CA; [3]Washington State Univ., Spokane, WA

Abstract: The prevalence of post-traumatic stress disorder (PTSD) is rapidly increasing due to the return of recent war veterans, but therapeutics used to treat PTSD are limited by extreme side effects or lack of efficacy. Current therapeutics act on broad targets; thus, there is a need to identify novel therapeutics that confer regional and molecular specificity to their treatment. Phosphodiesterase-4A (PDE4A) may allow for such specific regional and molecular

pharmacological targeting. PDE4A is a member of a family of enzymes that degrade cAMP, and is highly expressed in brain regions involved with anxiety, memory, and PTSD such as the hippocampus. PDE4A has six known splice variants which are created using alternative promoters and splicing, and have distinct N' termini resulting in targeted compartmentalization throughout the cell. Preliminary studies suggest knockout of PDE4A enhances fear memory; however, very little is known about how an experience such as fear-memory formation impacts PDE4A alternative splicing and subsequent splice-variant expression. The purpose of this study was to identify the effect of contextual fear conditioning (CFC) on PDE4A splice-variant expression, activity, and localization. Using RNA-Seq and exon-level analysis, we identified a specific intron in the PDE4A gene significantly elevated 30 minutes after CFC. Junction analysis reveals this intron is retained within distinct splice variants, and may be playing a regulatory role in PDE4A signaling. This is the first study to demonstrate behavioral-dependent increase of an intron, and may represent a novel mechanism for the regulation of PDE4A and fear memory.

Disclosures: R. Hansen: None. **S. Poplawski:** None. **G. Porcari:** None. **R. Havekes:** None. **L. Peixoto:** None. **T. Abel:** None.

Poster

728. Fear Memory: Molecular Mechanisms

Location: Hall A

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Program#/Poster#: 728.06/BB75

Topic: F.02. Animal Cognition and Behavior

Support: R21MH098506

Title: Deletion of PAC1 receptors from the medial intercalated cells of the amygdala enhances fear generalization and decreases the rate of fear extinction

Authors: *A. K. RAJBHANDARI[1], Y. HUANG[1], V. MAKHIJANI[1], J. WASCHEK[1], M. FANSELOW[2];
[1]Neurosci., [2]Psychology, Univ. of California-Los Angeles, Los Angeles, CA

Abstract: Post-traumatic stress disorder (PTSD) involves inappropriate inhibitory control over fear after exposure to life-threatening traumatic experiences. Previous studies have linked the neuropeptide pituitary adenylate cyclase activating peptide (PACAP) and its G-coupled receptor PAC1 to PTSD diagnosis and symptom severity. PACAP and PAC1 are expressed in the neural circuitry of fear and regulate conditioned fear behaviors. Using mice expressing green fluorescent protein (GFP) in PACAP containing neurons we found that PACAP-containing

neurons in the basolateral portion of the amygdala project into the medial intercalated cells (mICCs). mICCs are crucial for modulating fear extinction and express PAC1 receptors. Therefore, we investigated whether deletion of PAC1 receptors from the mICCs alters fear acquisition, generalization or extinction. In mice with floxed PAC1 gene, AAV-driven Cre-recombinase or GFP control was infused into the mICCs. After viral expression, mice went through a fear acquisition protocol (1 trial/day for 5 days) in which 4-minute after being placed into a conditioning context they were given a 0.65 mA, 1-second shock. PAC1 floxed mice that received Cre or GFP showed a similar acquisition rate and asymptote for fear learning. Mice were then placed in a different context to test fear generalization and their freezing behaviors measured for 4 minutes. PAC1 floxed mice with Cre showed enhanced freezing compared to mice with GFP. These mice also went through fear extinction in which they were placed in the acquisition context for 30 minutes every day and their fear behavior was measured from the first 4 minutes of the session. Rate of fear extinction was significantly decreased in PAC1 floxed mice with Cre compared to mice with GFP. Taken together, these results indicate that deletion of PAC1 receptors from the intercalated cells enhances fear generalization and reduces the rate of fear extinction potentially by decreasing feed-forward inhibition into the CeA. While these results are somewhat surprising given the putative role of PACAP in enhancing fear, they indicate that PACAP/PAC1 may play differential role in regulating fear depending on the site of action in the fear circuitry. The finding that the mICCs modulate fear generalization is a novel and interesting as studies have focused on the role of ICCs in fear extinction, but not generalization.

Disclosures: **A.K. Rajbhandari:** None. **Y. Huang:** None. **V. Makhijani:** None. **J. Waschek:** None. **M. Fanselow:** None.

Poster

728. Fear Memory: Molecular Mechanisms

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 728.07/BB76

Topic: F.02. Animal Cognition and Behavior

Support: NHMRC Grant

Title: BDNF val66met genotype enhances memory of fear and impairs extinction learning via glucocorticoid signaling

Authors: *M. NOTARAS[1], R. HILL[1], J. GOGOS[2], M. VAN DEN BUUSE[3];
[1]Florey Dept. of Neurosci. and Mental Hlth., Univ. of Melbourne, Melbourne, Australia;
[2]Columbia Univ., New York, NY; [3]La Trobe Univ., Melbourne, Australia

Abstract: Brain-Derived Neurotrophic Factor (BDNF) is a promoter of neuronal development and plasticity. The BDNF gene Val66Met polymorphism disrupts activity-dependent secretion of BDNF and has been associated with Post-Traumatic Stress Disorder (PTSD). However, despite being a requisite etiological factor, it has not yet been assessed whether prior stress exposure determines the expression or persistence of fear in Val66Met carriers. We therefore sought to model the long-term effects of chronic stress by using a novel Val66Met knock-in mouse that is genetically modified to express human BDNF (hBDNF) via endogenous mouse promoters. To simulate stress, corticosterone (CORT) was administered in the water of wild-type (hBDNF Val/Val) and mutant (hBDNF Met/Met) mice at a dose of 25mg/L from 6 to 9 weeks of age (adolescence). Following a two week wash-out period mice underwent behavioural testing and were screened for differences in spatial memory on the Y-Maze, memory of fear and extinction learning. At baseline, memory of contextual fear was disrupted in hBDNF Met/Met mice relative to hBDNF Val/Val controls but was rescued by a BDNF-CORT interaction which selectively enhanced the memory of hBDNF Met/Met mice. A similar phenotype was also observed on the Y-Maze, where hBDNF Met/Met mice showed a lack of preference for the novel arm over the other arms, but was reinstated to levels consistent with controls following the chronic CORT treatment. While extinction learning was unaffected in male and female hBDNF Met/Met mice at baseline, chronic CORT selectively attenuated the extinction learning of female hBDNF Met/Met mice during the late-phase of extinction trials. In summary, we report that chronic CORT exposure interacts with the BDNF Val66Met polymorphism to selectively modify the expression and extinction of fear. This novel gene-environment interaction highlights how fear is regulated by both BDNF and CORT exposure, providing mechanistic support for the treatment and prevention of stress-inducible psychiatric disorders with prominent sex-differences such as PTSD.

Disclosures: **M. Notaras:** None. **R. Hill:** None. **J. Gogos:** None. **M. van den Buuse:** None.

Poster

728. Fear Memory: Molecular Mechanisms

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 728.08/BB77

Topic: F.02. Animal Cognition and Behavior

Title: Opioid-dependent impairment in fear learning and extinction by prefrontal cortical stimulation

Authors: A. J. KIRRY, R. C. TWINING, E. M. DONCHECK, *M. R. GILMARTIN; Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Sustained firing in the prelimbic area of the medial prefrontal cortex (PL mPFC) of rats has been suggested to bridge the gap in trace fear conditioning (Gilmartin & McEchron, 2005; Gilmartin et al., 2013) and to drive freezing behavior to previously conditioned cues (Burgos-Robles et al., 2009). These findings support a general role for PL in the acquisition and expression of conditional fear. Here we sought to determine whether sustained optical stimulation of principal cells in PL would enhance fear acquisition and expression and impair fear extinction. Male Long-Evans adult rats were injected with a viral vector containing channelrhodopsin (AAV9-CamKiia-ChR2(H134R)-eYFP) or inactive control virus (AAV9-CAG-GFP) bilaterally into PL. To test whether stimulation of PL could enhance fear acquisition, laser light (473 nm, 20-Hz 10-ms pulses) was delivered during the 20-s trace interval separating the conditional cue and shock during trace fear training. To test whether stimulation of PL would drive the expression of fear and impair extinction, we delivered light during the cue on each trial of fear extinction. We found that PL stimulation did not enhance learning, but greatly impaired the acquisition of fear, reduced cued fear expression, and impaired extinction learning. Importantly, stimulation during the 20-s trace interval disrupted both cued and contextual fear associations, suggesting that stimulation may have interfered with the ability of the shock to drive learning. Follow-up experiments supported this possibility. Pre-shock stimulation of PL during unpaired conditioning impaired contextual and non-associative fear. Post-training inflation of fear by higher-intensity shock-alone trials in a novel context was attenuated by pre-shock stimulation of PL. A recent model of predictive fear learning implicates a role for PL in modulating ascending shock signals during the acquisition of fear (McNally et al., 2011), likely through opioid signaling in the periaqueductal grey (Fanselow & Bolles, 1979, McNally & Cole, 2006). Consistent with this model, we found that pre-training systemic administration of the opioid receptor antagonist naloxone (5 mg/kg, s.c.) prevented stimulation-induced impairments in context fear and partially restored cued fear learning in trace fear conditioning. Together, these results are consistent with a role for PL in top-down modulation of ascending pain signals necessary for fear learning and provide further evidence that the integrity of prelimbic trace interval firing is crucial for associating events separated in time.

Disclosures: A.J. Kirry: None. **R.C. Twining:** None. **E.M. Doncheck:** None. **M.R. Gilmartin:** None.

Poster

728. Fear Memory: Molecular Mechanisms

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Program#/Poster#: 728.09/BB78

Topic: F.02. Animal Cognition and Behavior

Support: National Institutes of Aging

Research Growth Initiative from UWM

Title: Delay fear conditioning enhances the intrinsic excitability of infralimbic neurons

Authors: *C. SONG, V. L. EHLERS, J. C. AITKEN, J. R. MOYER, Jr;
Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: The activity of medial prefrontal cortex (mPFC) is critical for fear memory expression through its reciprocal interaction with the amygdala (AM). Our recent data suggest that trace fear conditioning (where the CS and US were separated by a stimulus-free trace interval) enhances the intrinsic excitability of regular spiking infralimbic-AM (IL-AM) projection neurons but suppresses the regular spiking prelimbic-AM (PL-AM) projection neurons. Previous data also suggest that the brain circuits involved in trace fear conditioning are different from those of delay fear conditioning (where the CS and US were co-terminated). Thus, the current study was carried out to examine how acquisition of delay fear conditioning affects the excitability of mPFC neurons. Adult rats were randomly assigned into naïve, delay fear conditioned (COND), and delay fear conditioned-extinguished (EXT). On day 1, COND and EXT rats received one 4-trial session of delay fear conditioning. On day 2, EXT rats received one extinction session consisting of 10 CS-alone trials. On Day 3, all rats (naïve, COND, and EXT) received a brief CS-alone presentation to test fear memory. Within 1-hr following testing, brain slices were prepared and whole-cell recordings were made from layer 5 pyramidal neurons in both IL and PL. Analysis of data from 125 regular spiking neurons (71 IL, 54 PL) indicated that acquisition of delay fear conditioning significantly enhanced intrinsic excitability of IL neurons ($p < 0.05$). The excitability was still high following extinction but not significantly different from Naïve. In addition, fear conditioning was associated with an enhancement of hyperpolarization-activated current (Ih; $p < 0.05$), which remained high after extinction ($p < 0.01$). The excitability of regular spiking PL neurons was not significantly changed following conditioning or extinction. Thus, delay fear conditioning enhanced the intrinsic excitability and Ih of regular spiking IL neurons, which are consistent with our observations following trace fear conditioning. Interestingly, these observations are also consistent with a recent report that local blockade of NMDARs in IL reduces the amount of conditioned fear following both delay and trace conditioning, whereas local blockade of NMDARs in PL enhances the amount of conditioned fear following trace but not delay conditioning (Kwapis et al., 2014). Taken together, our data suggest that the activity of

IL pyramidal neurons facilitates the expression of both delay and trace fear memory, whereas the activity of PL pyramidal neurons suppresses the expression of trace fear memory.

Disclosures: **C. Song:** None. **V.L. Ehlers:** None. **J.C. Aitken:** None. **J.R. Moyer:** None.

Poster

728. Fear Memory: Molecular Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: Grants-in-Aid for Science Research on Innovative Areas (22115003; 25119004; 26250003)

Title: Experience-dependent modulation of fear conditioning

Authors: ***S. IWASAKI**, T. SAKAGUCHI, Y. IKEGAYA;
Lab. Chem. Pharmacol., Grad. Sch. Pharmaceut. Sci., Univ. Tokyo, Tokyo, Japan

Abstract: Fear may represent a protective response against danger. It is usually suppressed under normal situations, but anxiety disorders patients cannot regulate fear responses. One of the anxiety disorders is post-traumatic stress disorder (PTSD), which occurs following an unexpected exposure to a traumatic event. When people experience a traumatic event, some develop PTSD, but others do not. Recently, it is reported that one of the risk factors is a history of a brief trauma. However, it remains unknown how a previous trauma exposure enhances the sensitivity to a late-coming trauma. In this study, we used a mouse PTSD model and found that mice that had experienced a prior foot shock showed higher freezing responses. This prior shock-induced enhancement of fear conditioning (i.e., priming effect) lasted for 21 days. The priming effect was prevented by MK801, an N-methyl-D-aspartate receptor antagonist. Thus, this effect is dependent on neuronal plasticity. Other types of aversive stressors, such as forced swimming or tail pinch, did not enhance subsequent fear conditioning. Moreover, plasma corticosterone assays, elevated plus maze, and acetic acid-induced writhing test suggest that the priming effect did not result from an increased stress level, anxiety level, or pain sensitivity. These results suggest that a trauma was not generalized to modify the sensitivity to other trauma. Our study implies that a previous trauma induces specific long-lasting plasticity in its relevant neuronal pathway and thereby specifically enhances subsequent fear conditioning. The behavioral procedure used in this study may be a platform to elucidate the etiology of PTSD.

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Poster

728. Fear Memory: Molecular Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: Kansas State University USRG grant

Title: Individual differences in voluntary alcohol consumption predict fear incubation responses and fear acquisition in rats

Authors: ***A. PAJSER**, H. FISHER, M. GREER, P. KALLENBERGER, A. LIMOGES, C. LONG, C. L. PICKENS;
Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: PTSD and alcohol abuse frequently co-occur, but the cause of this co-morbidity is unclear. Alcohol exposure could alter problem drinkers' brains to promote the development of anxiety disorders, problem drinking could occur because of pre-existing anxiety (e.g.: self-medication), or a pre-existing factor could affect both anxiety and alcohol consumption. We studied the effects of chronic intermittent alcohol access (CIA) in rats from adolescence to early adulthood to determine if alcohol consumption alters or is correlated with conditioned fear responding after fear conditioning over-training, which models chronic stress. Male Long-Evans rats received CIA or water-only access for 6 weeks (PND 26-66). Rats were then food-restricted and began fear conditioning 15-16 days after the final alcohol access period. The rats were trained to lever-press and then received 10 days of fear conditioning. The rats were tested for conditioned fear (measured with conditioned suppression of lever-pressing) two days after the end of fear conditioning. Next, half of each treatment group received CIA access during a four-week fear incubation period; the other rats received only water during this time. Six days after the final day of alcohol access, the rats received a conditioned fear test. We found no effect or interaction of adolescent or adult alcohol access on conditioned fear. However, within the Adolescent Alcohol group, rats that had high levels of drinking during early adulthood exhibited lower conditioned fear both two days and one month after the end of fear conditioning. There was no correlation between alcohol consumption during the one-month incubation interval and conditioned fear in the one-month test. A follow-up experiment found that fear in a test 2 days after a single day of fear conditioning was not affected by or correlated with alcohol consumption, but high alcohol drinkers exhibited lower fear than low alcohol drinkers during the

fear conditioning session itself. Our results suggest that rats that consume higher levels of alcohol during early adulthood exhibit lower fear, which can be expressed during fear conditioning itself or during later tests. We found no evidence that alcohol consumption affected fear levels. This suggests that individual differences in the motivation to consume alcohol in early adulthood are correlated with fear acquisition or expression apart from any effects of alcohol consumption on the conditioned fear circuit. Future studies will investigate potential genetic differences or neural mechanisms in the brain that may be responsible for these differences in drinking and fear expression.

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Poster

728. Fear Memory: Molecular Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: NSFC Grant 31471079 to Yu Zhou

Title: Central activation of GHS-R1a in lateral amygdala blocks aversive memory formation by PLC/IP3/DAG and PI3K/Akt/mTOR pathways

Authors: M. YU, *Y. ZHOU, Q. ZHU, M. NIU, Q. KONG;
Qingdao Univ., Shandong, China

Abstract: Growth hormone secretagogue receptor-1a (GHS-R1a) is a highly conserved G-protein-coupled receptor (GPCR). Its broad distribution in the central nervous systems suggests that GHS-R1a signaling has important physiological functions beyond feeding control and energy metabolism. Our previous study showed that micro-infusion of ghrelin into the lateral amygdala (LA) activates GHS-R1a and interferes with the CTA memory acquisition in rats, however the underlying mechanisms are not known yet. In support of previous findings, we first showed here that local infusion of ghrelin into LA during CTA training inhibits Arc and zif268 mRNA expression in BLA triggered by memory retrieval. We further found that pre-infusion of either GHS-R1a antagonist YIL781, PLC inhibitor U73122 or PI3K inhibitor LY294002 into LA reverses ghrelin's blockade on CTA acquisition. YIL781, U73122 or LY294002 itself had no effect on CTA acquisition. Furthermore, IP3 receptor antagonist 2-APB, PKC inhibitor GF109203X or mTOR inhibitor rapamycin suppresses ghrelin's effect on CTA acquisition, while

MEK inhibitor, PKA inhibitor, D1 or D2 receptor antagonist does not. Those data suggests that PLC/IP3/DAG and PI3K/Akt/mTOR signaling cascades are two major downstream pathways mediating the modulatory effect of ghrelin/GHS-R1a activation on memory-related behaviors. Since both PLC and PI3K down-regulates sub-cellular concentration of PtdIns(4,5)P2 which is essential for the membrane docking and activity of inwardly rectifying potassium channes (Kir), we therefore tested the effect of Kir channel blockers ML133 hydrochloride and Tertiapin-Q on CTA acquisition. We found that both ML133 hydrochloride and Tertiapin-Q mimic ghrelin's blockade on CTA, while KCNQ channel blocker XE-991 and SK channel blocker apamin has no effect on CTA acquisition. All together, we concluded that ghrelin/GHS-R1a activation in lateral amygdala evokes PLC/IP3/DAG and PI3K/Akt/mTOR signaling and blocks aversive memory formation. PtdIns(4,5)P2 reduction caused by PLC and PI3K activation and subsequent Kir channels disfunction may also involved in this process.

Disclosures: **M. Yu:** None. **Y. Zhou:** None. **Q. Zhu:** None. **M. Niu:** None. **Q. Kong:** None.

Poster

729. Fear and Aversion Learning

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 729.01/BB82

Topic: F.02. Animal Cognition and Behavior

Support: PAPIIT IN212013

Title: Exogenous BDNF into adult neocortex strengthens a taste aversion memory

Authors: ***A. MARTÍNEZ**, L. F. RODRÍGUEZ DURÁN, M. L. ESCOBAR;
Facultad de Psicología, DIstrito Federal, Mexico

Abstract: Brain-derived neurotrophic factor (BDNF) is a neurotrophin that has been considered an important plasticity-related product as well as a potent molecular mediator for long-term memory. Our previous studies in the insular cortex (IC), a region of the temporal cortex implicated in acquisition and retention of conditioned taste aversion (CTA), demonstrated that BDNF is essential for the CTA consolidation. Recent studies show that BDNF-TrkB signaling is able to mediate the enhancement of memory. However, whether exogenous BDNF infusion is able to enhance aversive memories remains unexplored. In the present work, we administrated BDNF (2µg / 2µl per side) into the IC immediately after CTA acquisition in two different conditions: a “strong CTA” induced by 0.2M lithium chloride i. p., as unconditioned stimulus, and a “weak CTA” induced by 0.1M lithium chloride i. p. Our results show that BDNF converts

a weak CTA into a strong one, in a Trk receptor-dependent manner. We show for the first time that exogenous BDNF is able to increase an aversive memory-trace in the adult neocortex, supporting the view that BDNF expression is sufficient to strengthen memory-consolidation.
Supported by PAPIIT IN212013

Disclosures: **A. Martínez:** None. **L.F. Rodríguez Durán:** None. **M.L. Escobar:** None.

Poster

729. Fear and Aversion Learning

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Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI Grant 15K18348

Title: Long-term associative memory in Caenorhabditis elegans

Authors: S. ITO, *I. MARUYAMA;
Senior Investigator, Okinawa Inst. of Sci. & Technol. Grad. Univ., Okinawa, Japan

Abstract: Sensory neurons perceive information from the environment, which affects animal's behavior. *C. elegans* is an excellent model organism for the study of neuronal circuits that regulate the behavior, because of its relatively simple nervous system. The animal can also learn and store memory of non-associative memory like habituation and associative memory of two environmental stimuli. We are interested in molecular mechanisms and cellular networks underlying the associative memory, and have developed protocols for induction of short-term and long-term olfactory appetitive memories, STM and LTM, respectively, in *C. elegans*. We used 1-nonanol, a weak, volatile aversive chemical to *C. elegans*, as a conditioned stimulus (CS) and potassium chloride (KCl), a strong attractive substance, as an unconditioned stimulus (US). We conditioned animals with massed and spaced trainings to induce STM and LTM, respectively. Young adult animals in a small plastic tube sealed the bottom with a 30 um-nylon mesh sheet were stimulated with 1-nonanol vapor in a beaker, and then with KCl solution. For the massed training, animals were repeatedly conditioned as described above eight times without an inter-trial interval (ITI) between the conditionings. For the spaced training, on the other hand, animals were conditioned in the similar way to the massed training with ITI, during which animals were rested on an NGM plate. Immediately or hours later after the conditioning, animals were analyzed by chemotaxis assay on an agar plate spotted with 1-nonanol. As results, the trained animals successfully learned and retained the memory by the massed training and the

spaced training. We have also examined whether the training induces CS-specific memory by using 2-nonanone as a CS, instead of 1-nonanol. After training with 2-nonanone and KCl, animals formed 2-nonanone-specific, but not 1-nonanol-specific, associative memory. These results demonstrate that the protocols can successfully induce associative STM and LTM in *C. elegans*. We are currently trying to elucidate neuronal circuits responsible for the learning and memory.

Disclosures: S. Ito: None. **I. Maruyama:** None.

Poster

729. Fear and Aversion Learning

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Topic: F.02. Animal Cognition and Behavior

Support: FAPESP 11/08575-7

FAPESP 13/03040-3

FAPESP 13/03039-3

Title: Dopaminergic and Serotoninergic modulation on the avoidance response

Authors: *G. F. ANTUNES[1], C. C. OLIVEIRA[2], M. C. CASTRO[2], F. V. GOUVEIA[2], M. D. J. SENO[2], L. T. SANTOS[2], M. C. CARVALHO[3], M. L. BRANDÃO[3], E. T. FONOFF[2], M. J. TEIXEIRA[4], J. P. OTOCH[4], R. C. R. MARTINEZ[2];
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Abstract: Anxiety is understood as a body's response to situations in which the source of threat to the individual is not well defined. The two-way active avoidance test enables to understand the role of anxiety in the learning process. Avoidance is known to involve an instrumental response that modifies aversive, threatening emotional states and bodily responses. Two populations of animals have been distinguished in this test, the good avoiders and the poor avoiders. The poor avoiders show less than 20 avoidance responses and exhibited the freezing response. The dopamine and serotonin are important neurotransmitter activated during aversive stimulus. The aim of the work was to study the role of dopamine and serotonin as a modulator of two way

avoidance test. For that, first it was evaluated the role of dopaminergic (D1 and D2) and serotoninergic receptors (5-HT2C and 5-HT1A) on good performers in this test. Secondly, it was compared the amount of dopamine and serotonin in post-mortem amygdala comparing good and poor performers using High Performance Liquide Chromatography (HPLC) analysis. Wistar rats (n=77) have been trained in the avoidance test for 7 days. In the next day (Day 8) they received intraperitoneal injection of saline, D2 antagonist - Sulpiride (20 or 40 mg/Kg) or D1 antagonist - SCH 23390 (0.025 or 0.05 mg/Kg), or 5-HT2C antagonist - Kentaserina (1 or 2 mg/kg) or 5-HT1A antagonist - Way 100635 (1 or 2 mg/kg). On day 7, there was no difference in avoidance ($F(4,47)=0.33$ $p=0.85$. On day 8, after the administration of dopaminergic antagonist (day 8) there was a reduction in the number of avoidance response after D1 antagonist administration (SCH 0.05; 0.025mg/Kg) and D2 antagonist ($F(4,47)=15.11$, $p=0.00001$) in comparison with saline. The administration of 5-HT2C (Kentanserina 2mg/kg) and 5-HT1A(Way 1mg/kg) did not affect the number of avoidance response ($F(4,27)= 0.19$ $p= 0.99$). HPLC showed that there was no difference in dopaminergic content (Total DOPAC $F(2,53) = 1.82$ $p = 0.17$; Right amygdala DOPAC $F(2,25) = 1.50$ $p = 0.24$; Left amygdala DOPAC $F(2,25) = 1.25$ $p = 0.30$). HPLC showed an increased in serotoninergic content in the right side of amygdala in good performers in comparison with poor performers (amygdala Total HVA $F(2,53) = 0.89$ $p = 0.42$, HVA right amygdala $F(2,25) = 4.97$ $p = 0.02$; Left amygdala HVA $F(2,25) = 0.86$ $p = 0.44$). Thus, this approach suggests that dopamine and serotonin neurotransmitters may play important role in the avoidance response through D1 and D2 receptor and the amygdala is the main nucleus of serotoninergic modulation.

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Poster

729. Fear and Aversion Learning

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Alfred P. Sloan Research Fellowship

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Title: Striatal network organization predicts initial learning of Pavlovian associations

Authors: *K. BAKHURIN[1], V. MAC[2], P. GOLSHANI[2], S. C. MASMANIDIS[2];
[1]Neurobio., [2]UCLA, Los Angeles, CA

Abstract: Animals need to rapidly learn to guide and select actions that lead to positive outcomes. The striatum, as the main input structure of the basal ganglia, is thought to play a critical role in reward-guided action learning. We used state-of-the-art silicon probes with 256 channels to perform simultaneous recording of over 100 units in the striatum of awake behaving mice. Mice performed an odor discrimination task known to recruit striatal neuron activity in a learning-dependent manner. Populations of striatal medium spiny neurons (MSNs) that encoded information about cues and rewards (discrimination of reward- vs nonreward-predicting trials) were electrophysiologically distinct from their non-cue encoding neighbors, showing higher excitability, lateralized positioning in the striatum, and an increased likelihood of synchronous spontaneous activity. The functional segregation of cue-discriminating MSNs from the non-discriminating population was negatively correlated to behavioral performance in individual animals actively learning the task. However, in well-trained animals, network organization did not predict behavioral performance. Opportunities for new learning in the well-trained group reorganized the MSN network into states that resembled early phases of learning. These findings suggest that learning to link stimuli with behavioral responses benefits from a decorrelated striatum and that striatal networks reorganize to improve performance over time.

Disclosures: K. Bakhurin: None. **V. Mac:** None. **P. Golshani:** None. **S.C. Masmanidis:** None.

Poster

729. Fear and Aversion Learning

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Program#/Poster#: 729.05/BB86

Topic: F.02. Animal Cognition and Behavior

Title: Memory forgetting: exploring the mechanisms underpinning memory decay

Authors: *L. O. ALVARES, R. SACHSER, F. SANTANA, F. DUTRA, A. CRESTANI, J. QUILLFELDT;
Federal Univ. Rio Grande do Sul, Porto Alegre, Brazil

Abstract: Although the ability to retain and retrieve long-term memories is crucial to guide properly our behavior, most of the daily life experiences are forgotten overtime. Despite the fact that this process be the most common outcome in memory, the neural signature of forgetting remains largely unknown. In the current study we used behavioral, pharmacological and electrophysiological approaches to describe a novel mechanism that controls memory forgetting. First, using a hippocampus-dependent object location (OL) task, we determined when rats start to forget long-term memory (LTM) in a time-dependent manner. Then, we demonstrate that chronic inhibition of the (1) N-methyl-D-aspartate receptor (NMDAR), (2) L-type voltage-dependent calcium channel (LVDCC), and (3) protein phosphatase calcineurin (CaN), keep memory in a time-point in which it is ordinarily forgotten. Moreover, using a hippocampus-independent object recognition (OR) paradigm, we found that NMDAR also controls the decay of this type of information, suggesting that forgetting results from a well-regulated brain-wide active process mediated by NMDAR activation. We further demonstrate that intra-hippocampal infusion of ifenprodil after LTP induction *in vivo* prevents the depontentiation of CA1 field evoked plasticity, showing for the first time that downregulation of GluN2B-dependent signaling is critical to sustain LTP in a non-decaying state. Taken together, our findings indicate the existence of an active process that controls the synaptic plasticity responsible for LTM maintenance overtime.

Disclosures: L.O. Alvares: None. **R. Sachser:** None. **F. Santana:** None. **F. Dutra:** None. **A. Crestani:** None. **J. Quillfeldt:** None.

Poster

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Support: HHMI international predoctral fellowship

HHMI

Title: Separable memory formation and retrieval circuits generate aversive olfactory imprinting in *C. elegans*

Authors: *X. JIN, N. POKALA, C. BARGMANN;
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Abstract: Early life memory can be particularly stable and influential, but the sites and molecules that enable this privileged learning are not understood. Pairing olfactory stimuli with a nociceptive cue allows animals to associate aversion to the experienced odour. It has previously been reported that adult *C. elegans* can learn to avoid the pathogen Pseudomonas aeruginosa (PA14) after a 6-hour exposure, but lose the memory after 12 hours (Zhang et al Nature 2005). We have applied this aversive conditioning paradigm to newly hatched *C. elegans* larvae, and found that early pathogen experience generates long-term aversion behaviour, a process we call aversive imprinting. We found that imprinted animals specifically avoid pathogen odours for days after exposure, whereas training at any other stages cannot elicit such long-term memory. Through chemical-genetic neuron silencing, we identified two groups of neurons that act independently in memory formation and memory retrieval. The neuromodulator tyramine is required in the memory formation circuit during the learning phase. Multiple tyramine receptors are required for memory, suggesting broad circuit modulation by the learning signal. A G-protein coupled tyramine receptor is dispensable for adult aversive learning but required in one of the memory retrieving neurons for aversive imprinting, thus bridging the two subcircuits by linking tyramine production during learning to memory retrieval days later. The early imprinting experience modifies neuronal activity and output of the memory circuit, allowing animals to express their privileged memory by strategically turning away from the pathogen they experienced in the larval stage.

Disclosures: **X. Jin:** None. **N. Pokala:** None. **C. Bargmann:** None.

Poster

729. Fear and Aversion Learning

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Topic: F.02. Animal Cognition and Behavior

Support: NIDCD DC006456

Title: Anesthesia-induced conditioned taste aversions

Authors: *J. ARTHURS, J.-Y. LIN, S. REILLY;
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Abstract: We are interested in two types of taste learning: conditioned taste aversion (CTA) and taste avoidance learning (TAL). Each type involves a Pavlovian association between a taste (conditioned stimulus, CS) and postingestive consequences (unconditioned stimulus, US). What differentiates them is the nature of the learning. CTA involves a reduction in the hedonic value or palatability of the taste CS which determines intake whereas TAL causes a reduction of intake without a downshift in palatability (i.e., the taste CS becomes a signal for impending danger). Our laboratory has used lick pattern analysis (e.g., lick cluster size) to assess palatability and demonstrated that USs reported to cause TAL (e.g., amphetamine, gallamine, morphine) actually produce CTAs. The present experiments examined whether anesthetic drugs, thought to support TAL, may actually produce CTAs. In Experiment 1 male Sprague-Dawley rats were adapted to a water schedule allowing 15-min access in the morning (in drinking chambers) and afternoon (in home cages). Rats were assigned to three groups Group P0 (n = 10), Group P15 (n = 10), and Group P30 (n = 10), balanced for intake and lick cluster size. Conditioning trials consisted of replacing morning water with a 0.1% sodium saccharin CS followed 5-min later by an injection of the US. Group P0 was injected ip 1 ml/kg saline, Groups P15 and P30 were injected with, respectively, 15 and 30 mg/ml/kg of pentobarbital. On the following day control injections occurred 2-hrs after morning water access, Group P0 was divided into two sub-groups given either 15 or 30 mg/ml/kg of pentobarbital, while Groups P15 and P30 were injected with saline. A recovery day with regularly scheduled water access and no injections preceded the next conditioning trial. Two conditioning trials were followed by a CS-only test trial. Experiment 2 was conducted exactly like Experiment 1 except the US groups were K30 and K60 that received either 30/1.5 or 60/3.0 mg/ml/kg of ketamine/xylazine, respectively. In both experiments, each drug group showed significant decreases in both CS intake and lick cluster size (i.e., palatability), indicating the acquisition of CTAs. These findings expand the range of stimuli known to cause CTAs. Thus, our current model of CTA encompasses USs such as anesthesia, internal pain, and drugs of abuse, in addition to gastrointestinal malaise.

Disclosures: **J. Arthurs:** None. **J. Lin:** None. **S. Reilly:** None.

Poster

729. Fear and Aversion Learning

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NS48156.

Title: Conditioned inhibition changes in Hermissenda type A photoreceptors

Authors: *J. FARLEY;
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Abstract: Previously, we found that conditioned inhibition (CI)/safety signal learning in *Hermissenda* (*H.c.),* established by repeated explicitly-unpaired (EU) presentations of light (CS) and rotation (US), separated by a fixed ISI > 4.0 min, resulted in increased phototactic behavior and decreased photoresponses and spike activity of ocular Type B photoreceptors (Britton & Farley, 1999, *J Neurosci*; Walker et al., 2010, *Front Behav Neurosci*). Recent studies (Farley et al., 2015) have determined the ionic conductance changes that underlie the persistent decreases in B cell light-responses and spike frequencies due to EU training: increases in two somatic K^+ currents (I_A and $I_{K\text{-}Ca}$). Because both Type B and A cells undergo long-term neurophysiological changes due to CS-US pairings, these changes qualitatively oppose one another yet are complementary in their contribution to decreased phototaxis, it seemed likely that EU-produced decreases in B cell light responses might be accompanied by parallel *increases* in the responsiveness of A cells. Here, I report on the changes observed in both synaptically-intact spiking Type A cells, as well as those of synaptically-isolated non-spiking A cells, 1-3 days following behavioral training of intact animals. Animals were randomly assigned to one of 3 treatment conditions: EU, random (RDM), or untrained home cage controls (HC). In recordings from synaptically-intact A cells, resting R_{in} values were significantly greater for EU cells (n = 8) than for CON cells (n = 16, pooled results for RDM and HC cells, which did not differ), suggesting that one or more class of ion channels open at rest were shut by CI training. During repeated 30 sec light steps, EU Type A cells showed significantly greater light-evoked steady-state generator potentials (SSGPs) than CON cells, for all light steps and at all times within the light steps. Similarly, EU A cells generally spiked more frequently than CON cells. However, the magnitude of the spike frequency difference diminished within and across light steps. Because the photoreceptors in the *H.c.* eyes reciprocally inhibit each other, and EU B cells show decreased light responses and spiking, the increased light response and spiking of synaptically-intact A cells from EU animals could be due to either disinhibition from B cells (network effects) or alterations in intrinsic ion conductance systems. In recordings from synaptically-isolated A cells, the light-responses (SSGPs) were significantly greater for EU cells (n=10) vs. controls (n=8), and were apparent throughout all light steps. Thus, these results have identified a second site of intrinsic change and memory storage in *H.c.* underlying CI learning.

Disclosures: J. Farley: None.

Poster

729. Fear and Aversion Learning

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Topic: F.02. Animal Cognition and Behavior

Support: HHMI

Title: Aversive experiences transform amygdalar ensemble coding during fear learning

Authors: *B. F. GREWE[1], J. GRÜNDEMANN[2], J. LECOQ[1], L. KITCH[1], J. MARSHALL[1], J. PARKER[1], J. LI[1], A. LÜTHI[2], M. SCHNITZER[1];
[1]Stanford Univ., Palo Alto, CA; [2]FMI, Basel, Switzerland

Abstract: Learned emotions are fundamental aspects of human and animal behavior and are crucial for survival. The basal and the lateral amygdala (jointly abbreviated BLA) are necessary for emotional learning and link sensory-driven neural inputs to outputs controlling fearful and reward-seeking behaviors. Prior studies have uncovered molecular, synaptic and cellular substrates of fear memory in the BLA, but neural ensemble mechanisms underlying fear learning remain unknown. In our study we investigated neural population dynamics in the BLA by using a miniature (2 gram) fluorescence microscope [Ghosh et al. Nature Methods (2011) 8:871-8)] together with a chronic mouse preparation [Ziv Y et al., Nature Neuroscience (2013) 16:264-6] to track the calcium dynamics of ~150-200 individual neurons per mouse throughout a six-day fear-conditioning paradigm. This approach allowed us to follow monitor the neural ensemble representations of both neutral and aversive stimuli (tones and mild electric shocks). We found that initially neutral conditioned stimuli (CS) and unconditioned aversive stimuli (US) activated separate populations of sparse but intermingled BLA neurons. Paired presentations of a neutral CS and an aversive US initiated both down- and up-regulation of individual neurons' CS-evoked responses. Together, these bi-directional changes transformed the ensemble representation of an initially neutral CS into a response pattern more similar to that of the US. Overall, our results provide a first glimpse towards understanding associative learning at the level of large neuronal ensembles in the BLA. Further, our approach to tracking large populations of individual neurons in the amygdala opens the door to time-lapse imaging studies of neural coding in a brain structure that likely plays a central role in several psychiatric disorders.

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Poster

729. Fear and Aversion Learning

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Topic: F.02. Animal Cognition and Behavior

Support: NHMRC568872

Title: Learning to ignore: A fundamental role for the prelimbic cortex in down-regulating attention towards irrelevant cues during fear conditioning

Authors: *M. SHARPE[1], S. KILLCROSS[2];
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Sch. of Psychology, UNSW, Sydney, Australia

Abstract: The prelimbic (PL) cortex is argued to promote fear expression, at odds with appetitive research implicating this region in attentional processing. This theory has prevailed despite contradictory evidence resulting from manipulation of PL function in aversive conditioning. More specifically, pre-training lesions of the PL cortex have produced enhanced conditioning to a conditioning stimulus (CS; Morgan and LeDoux 1995), enhanced conditioning to a context (Morgan and LeDoux 1995; Lacroix et al. 2000), a decrement in CS conditioning (Lacroix et al. 2000), or have no effect (Holson 1986), whereas post-training inactivation reduces fear responding (Corcoran and Quirk 2007; Sierra-Mercado et al. 2010). In contrast, there is a long history of research in the appetitive literature which implicates the PL cortex in directing attention towards predictive cues (Birrell and Brown 2000; Marquis et al. 2007; Sharpe and Killcross 2014). We investigated whether a role for the PL cortex in attentional modulation could explain the discrepant findings in aversive studies. Firstly, we found that varying the degree of competition between contextual and discrete cues produced marked differences in the ability of animals with PL lesions to express fear towards a discrete CS. Specifically, a reduction of competition between contextual and discrete cues restored CS fear responding in animals with PL lesions, consistent with an attentional account as the need for attentional modulation towards the predictive CS is reduced when competition from contextual cues is low. Secondly, we tested whether PL inactivation would impact on the ability to change the degree of attention directed towards two discrete cues in an overshadowing procedure. During conditioning, two stimuli were presented simultaneously to form an audio-visual compound and two were presented as elemental stimuli all followed by footshock. When stimuli were subsequently tested individually under extinction, saline-infused animals exhibited lower levels of responding towards the cues that comprised the compound relative to those conditioned individually, an effect attributed to the down-regulation of attention towards the less salient element of the compound during conditioning (Mackintosh 1975). PL inactivation during conditioning abolished demonstration of

the overshadowing effect at test. However, PL inactivation during the test session itself did not impact on the ability to demonstrate this effect. These data call for a refinement of the role of the PL cortex in aversive conditioning, implicating the PL cortex in directing attention towards predictive cues during fear conditioning procedures.

Disclosures: M. Sharpe: None. **S. Killcross:** None.

Poster

729. Fear and Aversion Learning

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Support: NeuroTek Fund

AIHS

Title: A robotically controlled laser and real time subject tracking software for the study of approach and avoidance

Authors: *J. WILSON[1], M. KESLER[2], S.-L. PELEGRIN[2], L. KALVI[2], A. GRUBER[2], H. STEENLAND[2,1];
[1]Neurotek Innovative Technol., Toronto, ON, Canada; [2]Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: When a predator is at a distance there is no immediate danger so we can plan an escape. As that predator comes within striking distance we behave much differently, possibly attacking the predator or simply freezing (Griebel, Physiol. Behav. 1996, McNaughton and Corr, Neurosci. Biobehav. Rev. 2004, Fanselow and Lester, Evol. and Behav. 1988). To examine these behaviors, an interactive laser beam (green color) was mounted on a 2-dimensional gimbal. Under control of the experimenter at a console, the laser could be moved around an arena to interact with a rat. Software was used to simultaneously track both the rat and the laser beam. If the laser beam came within ~90mm of the rat, the rat would either receive a reward or a subcutaneous shock (via implanted electrodes). Animals that received rewarding electrical brain stimulation could learn to chase the laser beam, while animals that received aversive subcutaneous shock learned to avoid the laser beam. Animals that acquired the aversive conditioning did not show the full spectrum of behaviors related to being an animal of prey.

Thus, the neutral stimulus (laser beam) used in this paradigm simplifies the interpretation of the interaction between distance and threat.

Disclosures: **J. Wilson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Study designed to test technology. **M. Kesler:** None. **S. Pelegrin:** None. **L. Kalvi:** None. **A. Gruber:** None. **H. Steenland:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Study designed to test equipment.

Poster

729. Fear and Aversion Learning

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Topic: F.02. Animal Cognition and Behavior

Title: Goal-directed visuomotor learning and long-term memory of free-swimming zebrafish larvae is unveiled via high-throughput automated detection and analyses of hunting sequences

Authors: *A. M. LAMBERT[1], M. A. MASINO[2];
[1]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; [2]Neurosci., Univ. of Minnesota- Twin Cities, Minneapolis, MN

Abstract: Prey capture is a compound ethologically-relevant behavior that can be broken up into its component elements to serve as a neuroethological platform for investigating its sensory, sensorimotor, motor, neuromodulatory, engrammic, attentional, and decision-making neural components. Zebrafish larvae offer one of the best opportunities to comprehensively elucidate the many neural facets of prey capture. Zebrafish innately begin hunting at just 4 to 5 days post fertilization (dpf), but providing larvae with early hunting experience could influence later prey capture performance via either visuomotor learning and/or the obtained sustenance from prey consumption. To determine the influences of these factors, we developed high-throughput, automated detection and analyses of hunting sequences in free-swimming larvae. This unbiased approach was achieved via automated tracking of eye convergences, which is a reliable hallmark of prey detection and pursuit. Larvae provided previous experience hunting either large paramecia or small colpoda, 24 hours prior to testing, exhibited improved prey capture of paramecia at 7 dpf, compared to naïve counterparts. This experience-dependent potentiation of prey capture was not simply due to obtained sustenance from prey, because a daily regimen of colpoda from 4 dpf onward did not improve the survival rate compared to unfed counterparts, although a daily regimen of paramecia or 5% egg yolk did. Compared to naïve counterparts,

experienced larvae tested at 7 dpf exhibited: 1) a shorter latency (by ~10 min) to increases in eye convergence rate and initiation of hunting sequences following initial seeding of paramecia to the arena 2) a shorter latency (by ~150 ms) between execution of subsequent hunting maneuvers within a hunting sequence 3) a markedly better capture attempt success rate (by ~30%) 4) consumption of all prey items in half the time course of a 5 hour assay. Moreover, there was a strong negative correlation (R = -0.94) between capture attempt success rate and inter-maneuver durations, indicating that shorter latencies between hunting maneuvers is predictive of a greater precision in prey capture attempts. Many of these long-term enhancements at 7 dpf were recapitulated by providing an exclusive visuomotor training experience at 6 dpf by exposing larvae to artemia, which they actively hunted but were incapable of ingesting due to their considerably large size. Collectively, these results suggest that zebrafish larvae are capable of goal-directed visuomotor learning and long-term memory, the underlying genes of which could be isolated from high-throughput assays via our automated and unbiased hunting analyses.

Disclosures: **A.M. Lambert:** None. **M.A. Masino:** None.

Poster

729. Fear and Aversion Learning

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Topic: F.02. Animal Cognition and Behavior

Support: Sapienza Grant Anno 2014 - prot. C26A14SHMY

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Title: Neurogranin gene deletion increases stress responsivity in heterozygous mice

Authors: V. CESTARI[1,2], D. SARAULLI[2], S. FARIOLI VECCHIOLI[2], V. MASTRORILLI[2], F. D'ALESSANDRO[2,3], M. COSTANZI[2,3], S. DE MARCHIS[4], *R. VENTURA[5];
[1]Sapienza Univ. of Rome, Rome, Italy; [2]IBCN - CNR, Roma, Italy; [3]Lumsa Univ., Roma, Italy; [4]Univ. of Torino, Torino, Italy; [5]Univerity la Sapienza Rome, Rome, Italy

Abstract: Diathesis-stress models refer to the notion that psychological disturbances can result from the interaction between inherent vulnerability and environmental stressors. At present, genetic traits are widely considered to lie at one pole of the dyad, and specific gene variants are being continuously added to the ranks of putative predisposing factors that can determine, in conjunction with adverse life events, the onset of severe psychiatric disorders, such as

depression, anxiety and schizophrenia. Recently, evidence has been reported for genome-wide significant association between schizophrenia and a single nucleotide polymorphism (rs12807809) located upstream of the neurogranin (NRGN) gene. Based on its ability to bind calmodulin in a Ca2+-dependent manner, NRGN - a protein kinase substrate expressed in the cerebral cortex, hippocampus and amygdala - has been proposed as a major regulator of calmodulin activity in the postsynaptic compartment, likely involved in modulating synaptic strength and hippocampal learning and memory. Consistently, changes in synaptic plasticity, accompanied by significant impairments in hippocampus-dependent learning tasks, have been demonstrated to follow NRGN gene deletion in mice, along with further psychotomimetic abnormalities affecting anxiety-related and social behaviors. The present study aimed to test the hypothesis that NRGN gene deletion exacerbates mouse susceptibility to the behavioral effects of acute stress. To this aim, a fear conditioning procedure was selected, as an environmental stressor, which had proven ineffective in producing enduring behavioral alterations in wild-type mice, as assessed in the open field, dark-light emergence, forced swimming and social interaction tasks. The same procedure was subsequently adminis-tered to NRGN-knockout siblings, in both homo- and heterozygosis, which led to the following observations: (i) according to previous reports, (-/-) mice were significantly impaired in all tests, compared to wild-type mice, with constitutive behavioral abnormalities never worsened by exposure to the stressor; (ii) naive heterozygous mice performed as wild-type mice in all tests, but exposure to the stressor resulted in behavioral alterations comparable to those observed in homozygous counterparts. Thus, effects of stress exposure on heterozygous NRGN-knockout mice may constitute a model of gene × environment interaction, with useful implications for translational research on neuropsychiatric disorders. For all the experimental conditions, data on c-Fos expression in mouse hippocampus, amygdala, and prefrontal cortex are also provided.

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Poster

729. Fear and Aversion Learning

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Topic: F.02. Animal Cognition and Behavior

Title: The dynamic nature of systems consolidation: stress during learning as a switch guiding the rate of the hippocampal dependency and memory quality

Authors: *L. K. PEDRAZA CORREA, R. A. SIERRA ORDOÑEZ, F. ZACOUTEGUY, J. HAUBRICH, J. QUILLFELDT, L. ALVARES;
Biophysics Dept., Federal Univ. of Rio Grande Do Sul, Porto Alegre, Brazil

Abstract: Memories fade after a time point, becoming more schematic or abstract. The loss of contextual detail in memory may reflect a time-dependent change in the brain structures supporting memory. It is well established that contextual fear memory relies on the hippocampus for expression shortly after learning, but becomes hippocampus-independent at later time point, a process called systems consolidation. This time-dependent process correlates with the loss of memory precision. Here, we investigated whether the training intensity predicts the gradual decay of the hippocampal dependency to retrieve memory and the quality of the contextual memory representation overtime. We found that learning intensity modulates the progressive decay of the hippocampal dependency and memory precision. Strong training intensity accelerates systems consolidation and memory generalization in a remarkable timeframe match. The mechanisms underpinning such process are triggered by the glucocorticoid and noradrenaline released during training. These results suggest that the stress/arousal levels during emotional learning act as a switch, guiding the fate of memory quality. A moderate stress will create a detailed memory, whereas a highly stressful training will develop a generic gist-like memory.

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Topic: F.02. Animal Cognition and Behavior

Support: USAMRMC NO: 09284002

Title: Temporal transcriptome and epigenome changes in the brain and blood of mouse models simulating post-traumatic stress disorder

Authors: S. MUHIE[1], A. GAUTAM[2], N. CHAKRABORTY[2], R. HAMMAMIEH[2], J. MEYERHOFF[2], *M. JETT[3];
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Abstract: Hemi-brains were collected from mice exposed to modified social-stress (to simulate post-traumatic stress disorder) from 1 to 12 days. We carried out transcriptome profiling and genome-wide DNA methylation assays for each time point. We also did transcriptome profiling of blood, and of brain regions implicated in fear learning and fear memory consolidation (amygdala, hippocampus, medial pre-frontal cortex and ventral striatum), which were collected from mice exposed to either 5 or 10 days of social-stress. We identified greater numbers of differentially regulated genes in mice stressed for 3 or more days. Whereas, greater numbers of differentially methylated probes were observed at earlier time points (5 days and earlier) in stressed groups. The latter was not expected since DNA methylation is normally considered to be a slower process. Upon assessment of the global transcriptional binding of Creb1 using ChIP-on-chip tiling arrays, we observed reduced transcriptional activities of Creb1 in stressed groups as compared to controls. This could explain why many of its targets (nerve growth factors and transcripts implicated in fear extinction processes) were suppressed in both the hemi-brains as well as specific brain regions in the stressed mice. The time course trajectories of the correlated gene expression and DNA methylation patterns indicate that differentially changed transcripts and methylated regulatory regions at shorter stress durations (up to 7 days) were largely reported to be associated with behavioral responses (hyperactivity, fear response, circadian rhythm and cognition/learning), while longer stress durations (8 or more days) were largely reported to be associated with metabolic processes, immune response, and addiction and emotional behaviors. Our data suggest that the earlier occurrence of the epigenetic changes may be driving the ensuing changes of gene expressions, leading to responses which modulate behavioral disorders. This in turn might lead to metabolic disorders, immune dysfunction and cardiovascular problems. At the transcriptome and epigenome levels, behavioral responses seem to trigger the other comorbid conditions. A feedback loop is highly probable in these instances, as is shown in the case of inflammatory responses when comorbid conditions can aggravate anxiety disorders.

Disclosures: S. Muhie: None. **A. Gautam:** None. **N. Chakraborty:** None. **R. Hammamieh:** None. **J. Meyerhoff:** None. **M. Jett:** None.

Poster

729. Fear and Aversion Learning

Location: Hall A

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Program#/Poster#: 729.16/CC4

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Ability to discriminate between highly ambiguous contexts is dependent on the sensory modality of contextual cues

Authors: *R. J. BALOG[1], R. J. KEELEY[2], N. S. HONG[2], R. J. MCDONALD[2];
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Abstract: Interpretation of contextual cues associated with different outcomes increases in difficulty when the contexts are defined with common elements or overlapping features, increasing ambiguity. We were interested in manipulating the level of cue overlap utilizing multiple sensory modalities to alter context ambiguity. Various cues were manipulated in our discriminative fear conditioning to context paradigm to investigate the influence and saliency of visual, olfactory, geometrical, and tactile sensory information in this task. For each version of the discrimination task, each context was identical except in one sensory modality. The abundance of overlapping cues defining the context chambers should represent a difficult discrimination to resolve except by the appropriate interpretation of the predictive contextual cue and proper orthogonalization of the other context details. This task was hypothesized to be dependent on hippocampal function. Results show that discriminability was affected by which sensory modality or type of information that was used for the unique feature of the context with olfactory information providing the strongest disambiguation function and geometrical information the weakest. Using this paradigm, we also observed a novel defensive behaviour that emerges in situations where the discrimination is more challenging. Essentially, when the rats are in a testing situation in which the discrimination has been difficult to resolve they remove one paw from the steel rod floors or both paws. Presumably this behaviour is elicited to avoid shock exposure. This behaviour does not appear when the context discriminations are not ambiguous and uncertainty about where the aversive outcome will occur is clear. We are also examining the impact of complete hippocampal lesions on performance in these ambiguous discriminations. This work suggests that discrimination between ambiguously defined contexts depends on the sensory modality of the unique contextual cue available during training. The results also show that when rats have difficulty discriminating between contexts they show a defensive behaviour not seen in less ambiguous context discriminations, a response elicited by the subjects likely designed to avoid the full impact of the aversive event if it occurs. It is possible that hippocampal dysfunction may impair the acquisition or encoding of the configural representation of cues needed to accurately discriminate between ambiguous contexts.

Disclosures: **R.J. Balog:** None. **R.J. Keeley:** None. **N.S. Hong:** None. **R.J. McDonald:** None.

Poster

730. Decision Making: Neurocircuitry

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 730.01/CC5

Topic: F.03. Motivation and Emotion

Support: University of Oxford Christopher Welch Scholarship

University of Oxford Clarendon Scholarship

Title: Effort based decision making in people with motivational deficits

Authors: *C. LE HERON[1], S. MANOHAR[2], T. CHONG[1], A. BLAKE[2], M. JACKSON[3], M. HUSAIN[1];
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Abstract: Apathy, a debilitating syndrome of reduced motivation, is now conceptualized as a disorder of goal-directed behavior. Effective goal-directed behavior requires an action-outcome representation, a core component of which is the effort - or cost - involved in generating an action. The integration of action effort with outcome (reward) forms the core of effort-based decision making, and has been shown in humans to involve key basal ganglia and frontal regions, particularly ventral striatum and dorsal anterior cingulate cortex. Lesions to these regions in animal models reduce effortful behavior for reward, without altering rewards' hedonic value, mimicking the apathetic phenotype. To investigate whether effort-based decision making is impaired in people with apathy, we developed a decision task probing the influence of reward and physical effort on choice, and administered it to individuals with apathy and matched controls. The task comprised six reward and six effort levels, and the 36 combinations of these were evenly sampled across 5 blocks. Reward was presented as apples on a tree (each worth a monetary value), whilst effort was expended via a handheld dynamometer, normalized for each participant. Each trial consisted of an "offer" of reward (1-15 apples) in return for effort (14-80% of maximal voluntary contraction). If the participant accepted the offer, they squeezed the dynamometer to the required force level and were rewarded, whereas if they rejected it, they waited an equivalent time before moving on to the next offer. Apathy was defined using existing scales (Lille apathy rating scale and apathy evaluation scale). Behavioral performance was analyzed by comparing effort-reward functions between groups, and a logistic regression model of choice, including reward, effort and reward/effort interaction as predictor variables. Apathetic individuals showed a baseline bias towards rejecting offers and collected significantly fewer apples across the experiment. They showed a relative insensitivity to reward, requiring a higher reward to make a given effort. Intriguingly, their decisions were relatively insensitive to the effort cost and also showed a significantly reduced interaction of effort and reward compared to

controls, consistent with disruption of effort-based decision making. These findings demonstrate dysfunction of effort-based decision making systems is associated with reduced motivation and consequent goal directed behavior. They provide evidence of a mechanism by which behavioural apathy can occur, and suggest neuropharmacological modulation of this mechanism as a potential avenue of therapy for this condition.

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Poster

730. Decision Making: Neurocircuitry

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Program#/Poster#: 730.02/CC6

Topic: F.03. Motivation and Emotion

Support: NWO MaGW grant 2011 404-10-062

Donders Institute Top Talent

Title: Offline TMS-fMRI reveals integration of reward, task and response information across corticostriatal circuits

Authors: *M. VAN HOLSTEIN[1,3], M. FROBOESE[2], J. O'SHEA[4], I. TONI[2], E. AARTS[2], R. COOLS[2,5];
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Abstract: To respond appropriately to a constantly changing environment, cognitive processes and subsequent action selection should be informed by changes in potential gains, e.g. reward prospects. Processing of such goals from different levels occurs in anatomically and functionally segregated corticostriatal circuits. It has been previously shown that i) the ventromedial prefrontal cortex (vmPFC) and ventral striatum are involved in reward processing; ii) the dorsolateral prefrontal cortex (dlPFC), caudate nucleus and anterior putamen are involved in cognitive control (e.g. task-sets); and the premotor cortex (PMC) and posterior putamen are involved in response selection (e.g. stimulus-response mappings). However, to enable adaptive behavior, integration of these goals and their corresponding corticostriatal circuits is crucial.

Anatomical animal work has suggested that this integration between different corticostriatal circuits may occur at the level of the striatum. More specifically, integration is thought to occur in a unidirectional manner, with information being transferred from ventromedial to increasingly dorsal circuits. We aimed to assess in humans whether affecting neural excitability in reward-related cortical regions leads to a cascade of processes by which reward information influences motor responses in the motor putamen. To this end we combined offline transcranial magnetic stimulation (TMS) with functional MRI while 27 subjects performed a task-switching paradigm with a reward manipulation, which required integration of reward information with the selection of changing task-sets and adequate responses. We used a counterbalanced within-subject crossover design in which each subject received, on separate days, stimulation over each of the three task-related networks (vmPFC, dlPFC and PMC). As expected, stimulation of the vmPFC versus a no TMS baseline modulated activity in the posterior putamen in a task-related manner (i.e. as a function of reward, task, and response integration) relative to the other stimulation sites. In addition, a functional connectivity analysis confirmed a connection between the posterior putamen and regions within the reward network (e.g. anterior cingulate cortex and vmPFC) as a function of reward, task, and response integration. Congruous with anatomical evidence of integrated corticostriatal circuits in animals, we present evidence in humans showing that stimulation of the reward circuit during a task that requires integration of reward, task and response information can alter processing in a circuit further down the functional cascade, associated with response selection.

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Poster

730. Decision Making: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Support: NIMH (NAK: R01MH090134)

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NIDA (RZG: R21DA034954)

NWO Rubicon Fellowship (AZ: 446-14-015)

Title: Local network differences in reactive aggression measured with resting-state fMRI and graph theory

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Abstract: Reactive aggression is characterized by excessive anger expression and limited anger control. Even when unchallenged by a task or provocation, individuals with trait patterns of these anger characteristics are predicted to have differences in resting-state functional connectivity as compared to non-angry controls. Here we used graph theory, a mathematical approach for studying complex network properties, in a matched clinical sample (age, education, and race) of 12 individuals with reactive aggression (RA) in comparison to 12 low aggressive controls (LA) who completed resting-state functional magnetic resonance imaging and the State/Trait-Anger Expression Inventory (STAXI-II). As expected, groups differed in the *outward expression of anger* and *anger control* ($p<0.001$) subscales. We found decreased clustering coefficient (the number of connections directly connected to a node over the number of possible connections for that node), a measure of the probability of connectedness between neighboring nodes, in the Sensorimotor Network (pre-motor cortex, mid-cingulate cortex, paracentral lobule, and dorsal precuneus), and visual superior occipital cortex in RA relative to LA ($p<0.05$, FDR-corrected). Correlations across groups between the clustering coefficient connectivity properties of the Sensorimotor Network with *outward expression of anger* and *anger control* revealed that decreased local connectedness of the mid-cingulate cortex and paracentral lobule was linked to increased outward expression of anger (p-corrected=0.001), and decreased local connectedness of the paracentral lobule and dorsal precuneus was linked to lower anger control (p-corrected<0.005), (p-threshold=0.006; 4 regions × 2 trait variables). Disrupted local connectedness of the Sensorimotor Network with neighboring regions in RA hints at deficits in monitoring, planning and control of motoric responses, and may predispose individuals prone to reactive aggression to act upon less-integrated and less-regulated reactions to salient environmental cues. This is further suggested by the relationship between decreased local connectivity in this network and increased outward expression of anger and lower anger control. We also provide a graph theoretical framework for integrating findings from previous studies showing an association between higher precuneus activity and higher negative emotionality and lower self-control in individuals with high trait aggression. Overall, lower local connectivity of sensorimotor regions may be a contributing factor for greater expression of anger and lower anger control in reactive aggression.

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Poster

730. Decision Making: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Support: Sir Henry Wellcome Trust Fellowship WT101261MA

Wellcome Trust Centre for Neuroimaging

Title: The neural representation of optimal decision-making during human spatial forward planning

Authors: *R. KAPLAN[1,5,2], J. KING[3], R. KOSTER[1], D. BUSH[4], W. D. PENNY[2], N. BURGESS[4], K. J. FRISTON[2];
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Abstract: The hippocampus and medial prefrontal cortex (mPFC) are involved in planning optimal spatial trajectories, yet the computations behind this type of decision-making are unclear. We examined the neural processes that enable humans to infer the shortest path between a starting point and target location, particularly focusing on how that inference is modified by prospection. Subjects viewed mazes offering routes that varied in hierarchical depth (amount of choice points in the maze) and path length during separate fMRI and MEG experiments. We used Shannon entropy to quantify uncertainty about the shortest path length for a given maze - and the implicit computational complexity of selecting the shortest path. We constructed predictors of neuronal (fMRI/MEG) responses in terms of the entropy (uncertainty) of the choice probability in each trial. Medial prefrontal cortex (mPFC), medial temporal lobe (MTL), parietal midline, and angular gyrus BOLD responses and right MTL 3-5 Hz theta power were greater in low entropy (i.e. greater path length differences) planning trials. Conversely, responses in the dorsal anterior cingulate cortex/pre-supplementary motor area were greater in high entropy trials (i.e. smaller path length differences). Notably, 3-5 Hz right MTL theta phase coupling with mPFC and right angular gyrus correlated with planning trial entropy. We then examined whether

any brain regions selectively responded to entropy during forward planning, i.e. choosing between different paths at points further in the maze. We found that rostral dmPFC and lateral frontopolar cortex BOLD responses correlated with increasing forward entropy and that right MTL-mPFC theta phase-locking predicted forward entropy. In sum, we found BOLD responses for spatial planning that overlap with brain regions observed during value-guided choice, with spatial forward planning responses overlapping with regions observed during prospective choice. Furthermore, regions displaying BOLD responses to planning entropy also showed theta phase coupling with the MTL that predicted planning entropy. Our findings illustrate how distributed responses in the MTL and mPFC contribute to optimal decision-making during spatial forward planning.

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Poster

730. Decision Making: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Support: NIMH DIRP

Title: Dissociation of amygdala and dopamine contributions to exploratory decision making in rhesus monkeys

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Abstract: The amygdala and dopamine are both known to regulate learning to enable flexible affective responses when contingencies are extinguished or reversed. By comparison less is known about the roles of the amygdala and dopamine in managing the tradeoff between exploitation and exploration that underlies decisions between multiple choice options. Novelty seeking represents a specific case of the explore/exploit dilemma as animals explore novel and unfamiliar stimuli and environments in pursuit of potential reward. From this perspective the utility of exploring novel options can be formally expressed as a combination of its immediate expected value and the future expected value of actions that could be made after choosing the novel option and receiving feedback (i.e. an exploration bonus). Using a Markov decision

process (MDP) model to explicitly compute these value estimates, we compared to a group of unoperated controls (N = 8), the relative impact of bilateral excitotoxic lesions of the amygdala (N = 4) or systemic blockade of the dopamine transporter (N = 3) on the novelty seeking behavior of rhesus monkeys. During the task, the monkeys learned to choose between three, probabilistically rewarded images. Periodically one of the three choices was replaced with a novel image the monkey had not yet associated with reward. A finite state, discrete time, discounted MDP was used to derive immediate and future value estimates for each of the monkeys' choices and then used to predict the monkey's exploration of the novel choice options. We found that both amygdala lesions and systemic increases in extracellular dopamine heightened the selection of novel choice options. However, compared to controls, increased novelty seeking in the animals with amygdala lesions was related to their overweighting of the estimated exploration bonus ($p < .001$) and reduced weighting of an option's immediate value ($p < .001$). Thus, monkeys with amygdala lesions were more exploratory but learned less efficiently from reward feedback. By comparison dopamine levels had no effect on learning and instead caused a persistent overvaluation of novel choice options in terms of their immediate expected value ($p < .001$). Together the results from these two experiments suggest the amygdala is important for learning consistent statistical relationships needed to optimize exploratory behavior, whereas dopamine more generally biases exploration.

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Poster

730. Decision Making: Neurocircuitry

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Title: Why Adaptive Coding? Signal and noise in neural transmission and adaptive coding in economic choices

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Abstract: In the context of economic decisions, adaptive coding refers to the fact that neurons encoding the subjective value of a given option adjust their firing rate to the range of values available in the environment. Adaptive coding has been found in the orbitofrontal cortex (Padoa-Schioppa, 2009, Kobayashi et al 2010), amygdala (Bermudez and Schultz, 2010), anterior cingulate cortex (Cai and Padoa-Schioppa 2012), ventromedial prefrontal cortex and ventral striatum (Cox and Kable, 2013). Adaptive coding has also been observed in dopamine cells encoding reward prediction errors (Tobler et al, 2005). All these studies found that the neuronal activity slope is larger when the range of values is smaller, while the range of firing rates remains constant across different environments. Importantly, adaptive coding can introduce biases in decision making (Padoa-Schioppa and Rustichini, 2014). Thus a fundamental question is: What (if anything) does the choice system gain from adaptive coding? Here we show that the slope adjustment induces an improvement in the speed-accuracy trade-off, and we provide a quantitative estimate of this gain. The improvement is essentially because the process governing signal transmission across neuronal layers has a drift proportional to the firing rate of pre-synaptic cells, but standard deviation proportional to the square root of that firing rate. Everything else equal, and assuming a linear encoding of values, increasing the slope affords a faster and/or more accurate decision. The speed-accuracy trade-off is regulated by the stopping policy, which may depend on the environment and the details of the rules allocating rewards. We apply this general result to the several neural models of economic decisions, including the Drift Diffusion model (Ratcliff, 1978), the Leaky Competitive Accumulation model (Usher and McClelland, 2001), and several versions of the Pooled Inhibition model (Wang 2002; Wong and Wang, 2006). The general result that adaptive coding affords a better set of speed and accuracy is robust to the specific model, and we estimate this gain. We also show that in non-linear decision models the accuracy may be a non-monotonic function of the input slope. Thus the limited activity ranges typically recorded in orbitofrontal cortex and other brain regions may be set to optimize decisions, and not be due to intrinsic physiological bounds.

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Poster

730. Decision Making: Neurocircuitry

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NSF Grant SES-1357877 to AR

Title: A neuro-computational model of economic decisions

Authors: *C. PADOA-SCHIOPPA[1], A. RUSTICHINI[2];
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Abstract: Lesion studies and neurophysiology indicate that key aspects of economic decisions take place in the orbitofrontal cortex (OFC). Specifically, single-cell recordings in monkeys choosing between different juices identified in this area three groups of neurons: offer value cells encoding the value of individual goods, chosen juice cells encoding the binary choice outcome, and chosen value cells encoding the value of the chosen good. An important and open question is whether and how decisions could emerge from a neural circuit formed by these three populations. Here we adapted a biophysically realistic neural network previously proposed for perceptual decisions (Wang 2002; Wong and Wang 2006). The domain of economic decisions is significantly broader than that for which the model was originally designed, because offers vary independently of each other whereas coherence in the random-dot task is a 1D parameter: Yet the model performed remarkably well. The input and output nodes of the network (OV and CJ cells) were naturally mapped onto two groups of neurons in OFC (offer value and chosen juice cells, respectively). Surprisingly, the activity of interneurons in the network (CV cells) closely resembled that of the third group of neurons, namely chosen value cells. This resemblance reflect the fact that inhibitory interneurons in Wang's model receive the input from the two types of CJ cells. The model reproduced several phenomena related to the neuronal origins of choice variability including choice hysteresis, the "predictive activity" of chosen juice cells and the "overshooting" of chosen value cells (see Padoa-Schioppa, 2013). It also generated testable predictions on the excitatory/inhibitory nature of different neuronal populations and on their connectivity. Some aspects of the empirical data were not reproduced, but simple extensions of the model could overcome these limitations. These results render a biologically credible model for the neuronal mechanisms of economic decisions. They demonstrate that choices could emerge from the activity of cells previously identified in the OFC, suggesting that chosen value cells directly participate in the decision process. Importantly, Wang's model provides a platform to investigate the implications of neuroscience results for economic theory.

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Poster

730. Decision Making: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Support: NIDA Grant DA036534

Thomas H. Maren Fellowship

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Title: Neural activity in basolateral amygdala encodes reward magnitude and risk of punishment in a risky decision-making task in rats

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Abstract: Elevated levels of risk-taking behavior are characteristic of substance addiction, and have the potential to precipitate and exacerbate substance use. A potential treatment strategy for addiction could be to attenuate such maladaptive choice behavior, so as to mitigate drug-seeking and potential relapse. To realize this goal, however, a thorough understanding of the neurobiology underlying normal risk-taking behavior is required. Using a rodent model of risk-taking, in which rats choose between a small, "safe" reward and a large, "risky" reward accompanied by a variable probability of punishment, we showed previously that an intact basolateral amygdala (BLA) is critical for integration of risk- and reward-related information to guide adaptive risk-taking. In the present experiment, we evaluated how neural activity in the intact BLA encodes risk- and reward-related information during task performance. Rats were trained in a modified version of the risky decision-making task in which reward magnitude and probability of punishment (50%) were independently manipulated in separate blocks of trials. During stable performance, rats chose the large reward significantly more than the small reward and chose the reward associated with risk of punishment significantly less than the reward associated with no punishment. Two drivable microwire bundles were then implanted immediately above the BLA. Upon recovery, neural activity was recorded while rats were tested in the risky decision-making task. Initial analyses indicated that during reward magnitude trials, there was an increase in BLA activity in anticipation of the large reward and a decrease in activity in anticipation of the small reward relative to baseline. A difference was also observed during risk of punishment trials, such that BLA activity increased in anticipation of the risky reward and decreased in anticipation of the safe reward relative to baseline. Importantly, the reward magnitude associated with each choice during the risk of punishment trials was held constant, indicating that differences in firing were due to encoding of risk-related information. Together, these data suggest that BLA neurons track the most salient option during choice behavior. Future work will compare BLA neural activity in this task between drug-naïve and cocaine-exposed rats to determine whether dysfunctional BLA activity contributes to drug-induced maladaptive risk-taking behavior.

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Poster

730. Decision Making: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Title: A novel group of dopamine neurons encoding stable object value memories

Authors: *H. F. KIM, A. GHAZIZADEH, O. HIKOSAKA;
NIH, NEI, LSR, Bethesda, MD

Abstract: Midbrain dopamine (DA) neurons are thought to be critical for reward-value based learning by modulating synaptic transmission in the striatum. Using macaque monkeys, we recently showed that visual object-value learning occurred slowly and the value memory was retained stably in neurons in caudate tail (CDt), unlike neurons in caudate head (CDh) (Kim & Hikosaka, Neuron 2013). We then found that CDt receives DA inputs exclusively from caudal-dorsal-lateral region of substantia nigra pars compacta (cdlSNc), which is segregated from rostral-ventral-medial SNc (rvmSNc) projecting to CDh (Kim et al., Front Neuroanat 2014). These data suggest that cdlSNc-DA neurons guide learning and retaining of stable object values in CDt. To test this hypothesis, we examined DA neuronal activity in two stages: 1) object-value learning, followed by 2) passive viewing with no reward feedback. Both rvmSNc neurons and cdlSNc neurons learned to discriminate high- and low-valued objects. However, they behaved completely differently in the passive viewing task: rvmSNc neurons stopped responding to the objects, whereas cdlSNc neurons continued to respond to the objects differentially: excited by high-valued objects and inhibited by low-valued objects. rvmSNc and cdlSNc neurons showed similar spike shapes and firing patterns, which are characteristic of DA neurons. We then histologically reconstructed recording sites of cdlSNc neurons and found them within a cluster of tyrosine hydroxylase-positive neurons, suggesting that they are dopaminergic. Furthermore, these cdlSNc-DA neurons were activated antidromically by stimulation in CDt, indicating their axonal projections to CDt. Our data suggest that DA neurons localized in cdlSNc contribute to the learning and maintaining of object value memories in CDt neurons. Since CDt exerts strong disinhibitory-inhibitory effects on the superior colliculus through the substantia nigra pars reticulata (Yasuda & Hikosaka, J Neurophysiol 2015), this novel DA mechanism would enable automatic gaze orienting to high-valued objects.

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Title: Temporal backpropagation of basal forebrain reward-prediction-error signals underlies associative learning

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Abstract: Reward prediction error (RPE) signals have long been proposed in reinforcement learning (RL) theories as the driving force for new learning. A key prediction of RL theories that has rarely been observed experimentally is that, during learning, RPE signals should back-propagate from the unexpected outcome to the cue predicting that outcome through gradual shifts in latency. Here we provide evidence for such a temporal backpropagation in a novel RPE signal in the basal forebrain (BF). We first established that BF neuronal activity reflected RPE by encoding the difference between predicted and received reward. In rats exposed to a new stimulus-reward association, the receipt of unexpected reward following the new stimulus triggered a cascade of continuous backpropagation of BF activity to earlier events leading up to reward, which quickly emerged within a few trials and stabilized over several sessions. Backpropagated BF activity was tightly coupled with behavioral performance in single trials throughout learning. Furthermore, BF activity at an intermediate stage of RPE backpropagation, which was activated even in the absence of the new stimulus, underlied exploratory reward-seeking behaviors during the early learning phase in both stimulus-absent trials as well as in new stimulus trials. The evolution of BF RPE signals therefore reveals the underlying temporal dynamics of new learning, and also provides a rare glimpse into the internal models animals created to predict reward and guide reward-seeking behaviors.

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Poster

730. Decision Making: Neurocircuitry

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Topic: F.03. Motivation and Emotion

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Title: Topographic mapping of neocortical oscillations elicited by optogenetic stimulation of basal forebrain parvalbumin neurons

Authors: *E. HWANG[1], B. KIM[1], R. E. BROWN[2], T. KIM[3], J. T. MCKENNA[2], R. W. MCCARLEY[2], J. CHOI[1];
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Abstract: Particular behaviors are associated with different spatio-temporal patterns of cortical EEG oscillations. However, little is known about how subcortical projections to the cortex control these responses. GABAergic, parvalbumin-positive (PV+) projection neurons in the basal forebrain (BF) innervate GABAergic interneurons in multiple regions of neocortex and inhibitory neurons in thalamic reticular nucleus. Recent studies from our group (Kim et al., 2015, PNAS 112(11): 3535-3540) suggest that the BF PV projection to the cortex plays an important role in the state-dependent control of cortical oscillations, especially those in the gamma band (30-80 Hz). However, the cortical topography of this response is unknown. Thus, here we combined optogenetic stimulation of BF PV+ neurons with high-density (hd) EEG recordings in mice and determined for the first time the spatio-temporal frequency responses in the cortex. PV+ neurons were selectively targeted by double-floxed adeno-associated viral vector-mediated delivery of channelrhodopsin 2 to BF of C57BL/6 mice expressing Cre recombinase in PV+ neurons. hd EEG recordings were made using custom-made 32-channel EEG films laid over the skull. Optical stimulation at 10, 20, 30, 40 and 50 Hz was delivered via a patch cable connected to a 470-nm LED light source, while the mice were allowed to move freely in an unrestrained condition. Optogenetic stimulation of BF PV+ neurons at gamma band frequencies (30 and 40 Hz) induced preferential enhancement of frontal gamma band oscillations and increased frontal-frontal connectivity (N=3). On the other hand, stimulation at 10 and 20 Hz led to dominant

responses on centro-parietal and fronto-central regions, respectively. Thus, there were distinct topographical responses with varying stimulation frequencies. Anti-phasic oscillatory responses were observed between frontal and centro-medial areas only during stimulation with frequencies higher than 30 Hz raising the possibility of non-uniform mechanisms of coordinating cortical oscillation in a spatially distinctive manner by BF PV+ neurons. Our results supports the idea that increasing the activity of BF PV+ neurons may be a potential target for restoring abnormal frontal cortical gamma oscillations associated with disorders such as schizophrenia and Alzheimer's disease.

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Poster

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Topic: F.03. Motivation and Emotion

Title: Optogenetic dissection of motivational salience neuronal circuits in the basal forebrain

Authors: *A. SCAGLIONE, R. GREENFIELD, S.-C. LIN;
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Abstract: The survival of animals depends critically on prioritizing responses to motivationally salient stimuli. Recent studies have identified a group of neurons in the basal forebrain (BF) that encodes motivational salience of attended stimuli using robust bursting responses (Lin & Nicolelis, 2008). Such salience-encoding BF responses generate an event-related potential response in the frontal cortex (Nguyen & Lin, 2014) and lead to faster decision speed (Avila & Lin, 2014). However, the neurochemical identity of salience-encoding BF neurons remains unknown. Determining their neurochemical identity is a key step toward understanding their circuit-level functions especially because BF is an anatomically complex and heterogeneous region, comprised of multiple spatially overlapping macrosystems and several distinct neuronal populations. The goal of this study is to determine the neurochemical identity of salience-encoding BF neurons by optogenetically tagging and recording the three major cortically-projecting BF neuronal populations in freely behaving mice. To achieve this goal, we first established an experimental platform in three strains of transgenic Cre mice (PV-, ChAT- and VGluT2-Cre) that combines operant behavior, electrophysiology and optogenetics. Mice were trained to maintain fixation in a nosepoke port and then respond quickly to a reward-predicting

tone to collect reward in an adjacent port. Mice were able to achieve high hit rates (>90%) and high trial numbers in a session (>150 rewarded trials) with fast reaction times (<300ms). Neuronal recording in combination with photo-stimulation of the BF show that: 1) The majority of recorded BF neurons in mice showed robust bursting responses to the motivationally salient tone similar to salience-encoding BF neurons in rats and 2) photo-stimulation of the three major cortically-projecting BF neuronal populations can directly or indirectly modulate (excite or inhibit) bursting responses to the salient tone. Taken together, these results suggest that the three main populations of neurons participate in concert, directly or indirectly, in the circuit underlying motivational salience in the BF.

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Title: Neuroanatomical investigation of basal forebrain glutamatergic neurons using vGlut2-tdTomato mice

Authors: *J. T. MCKENNA[1], M. GAMBLE[2], C. YANG[1], J. M. MCNALLY[1], A. HULVERSON[2], S. WINSTON[1], S. THANKACHAN[1], R. W. MCCARLEY[1], R. E. BROWN[1]; [1]Research/Psychiatry, VA Boston Healthcare/Harvard Med. Sch., Brockton, MA; [2]Stonehill Col., Easton, MA

Abstract: Basal forebrain (BF) neurons play a crucial role in cortical activation, attention, and sleep-wake behavior. Of the three major BF neurotransmitter classes, glutamatergic neurons are the least well understood due to difficulties in identification. Thus, here, we validate a novel

transgenic mouse model that expresses a red fluorescent protein (tdTomato) in BF glutamate neurons. Vesicular glutamate transporter, subtype 2 (vGluT2)-tdTomato mice were created by crossing vGluT2-Cre Recombinase mice with a Cre-reporter strain expressing tdTomato. This mouse model allows online identification and combinatorial approaches with other transgenic mouse lines or viral vectors utilizing green/yellow fluorescent proteins. Immunohistochemical staining was performed against choline acetyltransferase (ChAT, synthetic enzyme for acetylcholine); parvalbumin (a subset of GABAergic neurons); and calbindin, to identify putative cortically-projecting neurons. The distribution of tdTomato+ neurons in the BF of vGluT2-tdTomato mice resembled that of BF vGluT2+ neurons identified previously by means of *in situ* hybridization. tdTomato was not expressed in cholinergic (N=3, 3154 vGlut2-Tom+, 1903 ChAT+ neurons analyzed) or parvalbumin (N=3, 3049 vGlut2-Tom+, 3528 PV+ neurons analyzed) neurons. 25.9±3.3% (ave±SEM) of BF vGluT2-Tomato neurons contained calbindin (N=3, 2872 vGlut2-Tom+ neurons analyzed). vGlut2-tdTomato mice were also crossed with GAD67-GFP knock-in mice to test for co-localization in GABAergic neurons. tdTomato was not expressed in GABAergic neurons (N=1, 1172 neurons analyzed). To investigate BF vGluT2 projections, floxed adeno-associated viral vectors (AAV) expressing ChR2-EYFP (Enhanced Yellow Fluorescent Protein) were unilaterally injected into BF. Efferent projections were identified in cortical regions, thalamus, and hypothalamus, as well as fibers apposed to BF cholinergic and PV neurons. BF vGluT2 neurons may modulate cortical activity through both direct cortical projections and interactions with BF cholinergic and parvalbumin neurons. Validation of this mouse model allows further study of the role of BF glutamatergic neurons in sleep-wake behavior, using *in vitro*, *in vivo* optogenetic, and pharmacogenetic approaches.

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Poster

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NHLBI HL095491

Title: Basal forebrain vGluT2-positive neurons: electrophysiological properties and cholinergic modulation

Authors: *C. YANG, J. T. MCKENNA, R. E. BROWN;
Psychiatry, VA Boston Healthcare Syst. and Harvard Med. Sch., Brockton, MA

Abstract: Basal forebrain (BF) wake-active neurons play an important role in cortical activation, attention and cortical plasticity. Among the three major types of BF cortically-projecting neurons (cholinergic, GABAergic and glutamatergic), glutamatergic neurons are the least understood, due to difficulties in identification. Here, we take the advantage of a novel transgenic mouse model [vesicular glutamate transporter 2 (vGluT2)-tdTomato mice] expressing a red fluorescent protein in the major group of BF glutamatergic neurons to characterize for the first time the intrinsic membrane properties and neurotransmitter modulation of identified BF glutamatergic neurons. Whole-cell, patch-clamp recordings were made from vGluT2-positive neurons in coronal brain slices. Most of these neurons were small/medium sized (Mean long-axis diameter: 14.6±0.8 μm, n=19). Most vGluT2 neurons were silent at rest (13/19) and had a maximal firing frequency of ~40 Hz i.e. they had a maximal firing frequency which is larger than that of BF cholinergic neurons but smaller than that of large-sized GABAergic/parvalbumin neurons. We categorized vGluT2 neurons into two groups: medium-sized (>15 μm) and small-sized (≤ 15 μm). Medium-sized vGluT2 neurons were located in HDB/SI/LPO regions of BF (5/6) and were strongly hyperpolarized (-17.5 ± 2.2 mV, n=5/5) by the cholinergic agonist, carbachol (50 μM) in the presence of 500 nM tetrodotoxin. Most small-sized vGluT2 neurons were located in the lateral MCPO or HDB/LPO (9/13) and did not show a postsynaptic response to carbachol (n=2). Our results suggest that BF glutamatergic neurons have distinct intrinsic membrane properties from BF cholinergic and GABAergic neurons. Unlike BF GABAergic neurons, glutamatergic neurons are hyperpolarized by cholinergic inputs, which may facilitate burst firing of these neurons during wakefulness and REM sleep via de-inactivation of low-threshold calcium currents.

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Poster

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Topic: F.03. Motivation and Emotion

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Title: Decoding the value related signal represented in the multiple areas of the prefrontal cortex using the ECoG electrodes

Authors: *S. TANAKA[1], K. KAWASAKI[2], I. HASEGAWA[2], T. SUZUKI[3], M. SAKAGAMI[1]; [1]Tamagawa Univ. Brain Sci. Inst., Machida, Tokyo, Japan; [2]Dept. of Physiol., Niigata Univ. Sch. of Med., Niigata, Japan; [3]Natl. Inst. of Information and Communications Technol., Ctr. for Information and Neural Networks, Osaka, Japan

Abstract: Decision making is one of the important mental processes. For making a decision between multiple options, sometimes the values of the options are used. Wide-ranging areas of the brain are involved in this economic decision making process. Especially, a lot of studies show that areas in the prefrontal cortex (PFC) play important but different roles for value calculation and comparison. Because of the different rolls of the areas in the PFC, the value signal should be reciprocated within areas in the PFC. However few studies directly examined the signal transmission between multiple areas of the PFC. Here, we tried to reveal the dynamic mechanism of value signal transmission in the wide-ranging areas of the PFC. A monkey was trained to perform a free-choice task. After start cue presentation monkey pressed a button then a hold cue appeared. While the monkey kept pressing the button, two visual cues which indicated the kinds of juices were presented sequentially with a short blank. Finally, two cues were presented simultaneously and the monkey touched one of them to obtain juice reward. From the choice behavior between two alternatives, the values of the rewards could be estimated. While the monkey performed this task, the local field potentials (LFPs) were recorded from electrocorticographic (ECoG) electrodes implanted on the left lateral prefrontal cortex (LPFC), the left orbitofrontal cortex (OFC) and both side of the medial prefrontal cortex (MPFC) of a macaque monkey. To examine whether the value of the reward is represented in 3 areas of the PFC, we tried to decode the values of the juices from the signal during 1st cue presentation for each area separately. We calculated the power in six frequency domains (δ, θ, α, β, *low-*γ, *high-*γ) and extracted the value related features which showed correlation to the value of the reward estimated from the behavior. With this extracted features, we tried to decode the value of the reward with Sparse Linear Regression (SLiR) algorithm. The decoded values calculated from 3 areas were highly correlated with the estimated value. The R^2 score was biased to be larger than 0 indicating a good decoding performance. We also performed ROC analysis to examine whether the decoder could discriminate most preferred juice and least preferred juice. Area under the ROC value was significantly larger than 0.5 and close to 1 indicating nearly perfect discrimination ability. Furthermore, the time windows, from which the features used to decode the value were selected, of MPFC was earlier than that of LPFC and OFC. From these results, we suggest that the value signal exists in 3 areas of the PFC and the temporal stage for value calculation were different.

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Topic: F.03. Motivation and Emotion

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Title: The neural basis of self-initiated movements in the larval zebrafish

Authors: ***A. JOUARY**[1], S. MEHYAOUI[2], G. SUMBRE[1];
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Abstract: Animal behaviors can be induced by sensory stimuli or reflect intentional self-initiated motor patterns. In the absence of salient sensory cues, the larval zebrafish spontaneously produces stereotypical tail movements similar to those produced during goal driven navigation. Our goal is to understand the mechanisms by which sequential activations of neuronal assemblies across different brain region predict the onset of a specific tail movement. In head-restrained larva, we simultaneously recorded tail movements and neuronal activity from a large portion of the brain using light- sheet microscopy (~5000 neurons at 10Hz).The tail movements were categorized according to stereotypical maneuvers using an unsupervised classifier. We identified spatio-temporal features of the neural activity predicting the onset of a specific tail movement around 1 sec before this onset. Moreover, preliminary analysis suggests that neurons whose activity predicts the occurrence of a tail movements are distinct and operate on shorter time scale than neurons predicting the categories of movements. This suggests that parallel pathways are coding the initiation and selection of the type of upcoming movement.

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Title: Prediction of rat lever pressing based on hippocampal theta oscillation

Authors: *N. TANAKA[1], K. SANO[2], R. MIYATA[4], T. AONISHI[5], G. CAPI[3], K. USUI[1,2], S. KAWAHARA[1,2];
[1]Grad. Sch. of Innovative Life Sci., [2]Lab. for Brain and Neural Systems Eng., Grad. Sch. of Sci. and Eng. for Edu., [3]Intelligent Robotics Lab., Dpt. Electrical and Electronics Systems Eng., Fac. of Eng., Univ. of Toyama, Toyama-shi, Japan; [4]Lab. for Mathematical Eng., Dpt. Mechanical Systems Eng., Fac. of Eng,, Univ. of Ryukyus, Nakagami, Japan; [5]Lab. for Theoretical Complex Systems, Interdisciplinary Grad. Sch. of Sci. and Eng., Tokyo Inst. of Technol., Yokohama, Japan

Abstract: Decision-making is one of the most complicated cognitive processes that require integration of neural activities across several brain regions. In the present work, we analyzed hippocampal local field potential (LFP) during a lever-pressing task, in which restrained rats pressed the right or left lever with their right or left hand, respectively, to control an e-Puck mobile robot that brought them a small amount of food. We found a predominance of theta range oscillation (4-8 Hz) in the hippocampal LFP, which began 1 s before the lever press and lasted for 2-3 s. The time course of theta convergence was similar between the right and left lever-pressings, suggesting that this convergence was not directly implicated to the motor output. Interestingly, we found stark differences in some behavioral and LFP parameters between earlier and later periods of a daily session, which were thought to correspond to hungry and satiated conditions in rats, respectively. As expected, the rats pressed the lever more frequently in the earlier period. Along with this, peak frequency of the theta oscillation significantly changed from 7.2 to 7.8 Hz, while total amount of the theta power (4-8 Hz) did not change. The peak frequency shift of hippocampal theta was also reported during decision-making epoch for the right-left selection in plus maze task and hence likely to reflect a change in behavioral state of the rat. Based on these results, we developed a method to predict the lever-pressing using the spectrum pattern of hippocampal LFP and the theta peak frequency. Using these parameters as features, we run the machine-learning algorithm and calculated cross-correlation between the template and temporal pattern of relative theta power. We found spectrum pattern was useful to predict the lever-pressing in this kind of cognitive effort-demanding task.

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Poster

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William N. & Bernice E. Bumpus Foundation (RRDA Pilot: 2013.1)

Title: A medial prefrontal-striosome circuit is selectively engaged by cost-benefit conflict decision-making

Authors: *L. G. GIBB, A. FRIEDMAN, D. HOMMA, K.-I. AMEMORI, S. J. RUBIN, A. S. HOOD, M. H. RIAD, A. M. GRAYBIEL;
MIBR/BCS, MIT, Cambridge, MA

Abstract: The striosomal compartment of the striatum can be distinguished from the surrounding matrix based on patterns of gene expression and connectivity, and imbalances between striosomes and matrix occur in neurological disorders. The function of striosomes is a decades-old mystery, but their connectivity suggests a function related to mood and motivation. Here, we developed a method for identifying, in tetrode recordings from the dorsomedial striatum of rats, the putative striosomal spiny projection neurons (SPNs) that preferentially receive input from a region of the rodent prefrontal cortex called the prelimbic cortex (PFC-PL). Our method is based on the response pattern of putative striosomal SPNs to electrical stimulation of the PFC-PL, which we validated in a set of rats by determining the positions of tetrode tips relative to striosomes in 3D reconstructions from immunofluorescently stained sections post-stimulation. We analyzed the activity of putative striosomal SPNs in five T-maze tasks: a cost-benefit conflict (CBC) task, in which the rat had to accept high cost in order to receive high reward; two versions of a benefit-benefit task, in which the rat chose between two rewards, either similar or dissimilar; a non-conflict cost-benefit task, in which high reward was paired with low cost; and a cost-cost task, in which the two options had equal rewards but different costs.

Strikingly, the activity of striosomal SPNs during the period between starting cue (click) and turn was low in the CBC task and high in the other four tasks. By contrast, SPNs not identified as striosomal, which based on the relative volumes of the striatal compartments should largely consist of matrix SPNs, were active in the click-to-turn period in all tasks. The behavioral results of optogenetic manipulation of the PFC-PL input to striosomes showed similar CBC selectivity. Optogenetic inhibition predominantly affecting PFC-PL input to striosomes had a large effect on decision-making only in the CBC task, increasing the animals' choices of the high-cost, high-benefit option, whereas optogenetic excitation of the same input increased the animals' choices of the low-cost, low-benefit option, again only in the CBC task. Optogenetic inhibition of the input to matrix from the anterior cingulate cortex significantly shifted the animals' choices toward the higher reward in all tasks having unequal rewards. Logistic modeling of the data suggested the possibility that optogenetic manipulation the cortico-striosomal circuit modulated sensitivity to cost selectively in the CBC task, whereas optogenetic manipulation of cortical input to matrix modulated sensitivity to benefit in all tasks.

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Poster

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William N. & Bernice E. Bumpus Foundation (RRDA Pilot: 2013.1)

Title: Striatal high-firing interneurons mediate inhibitory prefrontal-striosomal signaling during cost-benefit conflict decision-making

Authors: *A. FRIEDMAN, D. HOMMA, L. G. GIBB, L. G. GIBB, K.-I. AMEMORI, S. J. RUBIN, A. S. HOOD, M. H. RIAD, A. M. GRAYBIEL;
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Abstract: The functions of cortical inputs to the striosomal compartment of the striatum are unknown. Here, we compared the activity patterns of striosome-projecting prefrontal cortical neurons and striosomal spiny projection neurons (SPNs) in rats across five T-maze decision-making tasks: a cost-benefit conflict (CBC) task, in which a high-reward option is paired with high cost; two benefit-benefit tasks with similar or dissimilar rewards; a non-conflict cost-benefit task, in which the high-reward option is paired with low cost; and a cost-cost task with equal rewards but different costs. We antidromically identified putative striosome-projecting neurons of rodent prefrontal cortex in the region called the prelimbic cortex (PFC-PLs neurons) and orthodromically identified putative striosomal SPNs. Strikingly, we found that the activity patterns across tasks of PFC-PLs neurons and striosomal SPNs were complementary: In the period between the start cue and the turn, PFC-PLs neurons had high activity in the CBC task but low activity in the other tasks, whereas striosomal SPNs had low activity in the CBC task and high activity in the other tasks. Since the corticostriatal projection from PFC-PLs neurons should be excitatory, we hypothesized that the complementarity of the PFC-PLs and striosomal SPN patterns could be due, at least in part, to feedforward inhibition via striatal interneurons. Thus we examined the activity of high-firing neurons (HFNs, putative inhibitory interneurons) that we identified on the basis of spike and firing characteristics. We found that HFNs had burst-like episodes and that the within-burst firing rate pattern of HFNs across tasks was remarkably similar to the pattern of PFC-PLs neurons. Moreover, the firing rates of striosomal SPNs were negatively correlated with the within-burst firing rate of HFNs. These results emerged only when we used the within-burst firing rate of HFNs, suggesting that phasic, rather than tonic, activity of HFNs may be essential for the feedforward inhibition. Also consistent with the hypothesis of feedforward inhibition, SPN spikes aligned to the time of peak firing rate of simultaneously recorded HFNs exhibited inhibition, and the response latency of HFNs to electrical stimulation of PFC-PL was ~3 ms shorter than that of striosomal SPNs. When the corticostriatal axons were optogenetically inhibited in conjunction with electrical stimulation of PFC-PL, striosomal SPN activity decreased, whereas HFN activity increased. Our results suggest that the PFC-PLs pathway to striosomes is selectively engaged in CBC decision-making and that PFC-PL activation inhibits striosomal SPNs via striatal HFNs.

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Poster

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William N. & Bernice E. Bumpus Foundation (RRDA Pilot: 2013.1)

Title: Compartmental selectivity of a prefronto-striosomal pathway controlling decision-making under motivational conflict

Authors: *D. HOMMA, A. FRIEDMAN, L. G. GIBB, K.-I. AMEMORI, S. J. RUBIN, A. S. HOOD, M. H. RIAD, A. M. GRAYBIEL;
MIBR, MIT, Cambridge, MA

Abstract: Striosomes form a labyrinthine striatal compartment originally distinguished from the surrounding matrix by neurochemical properties. Although parts of the striosomal compartment are anatomically connected with regions related to motivation and emotion, the functions of striosomes are virtually unknown. Here, we developed rat models for optogenetic manipulation of striosome- or matrix-selective corticostriatal inputs. We verified the selectivity of virally labeled projections to striosomes and matrix, respectively, from zones within the prefrontal regions called in rodents prelimbic cortex (PFC-PL) and anterior cingulate cortex (PFC-ACC) by using immunofluorescence histology, confocal imaging, 3D reconstruction and densitometric analysis of corticostriatal axons in the region below the optical fiber tips. In this analysis, we used three different models to estimate the effect of optogenetic manipulation of these corticostriatal pathways. We found that the density of PFC-PL input labeling was ~5.2 times greater in striosomes than in matrix and that of PFC-ACC input labeling was ~2.6 times greater in matrix than in striosomes. We optogenetically manipulated these selective inputs in rats performing five T-maze-based decision-making tasks that we developed. Our tasks allowed us to measure quantitatively the motivation to approach reward and the motivation to avoid cost under different levels of motivational conflict. The rats' sensitivity to reward was measured by a benefit-benefit task in which different levels of benefit (chocolate milk concentration) were provided at each end-arm. The sensitivity to cost was measured by a cost-cost task in which the same benefit was given on both sides but one was coupled with higher cost (brighter light). To

investigate how the rats integrate cost and benefit under different levels of motivational conflict, we introduced a cost-benefit conflict task in which higher benefit was coupled with higher cost, and a non-conflict cost-benefit task in which higher benefit was coupled with lower cost. Optogenetic inhibition of the striosome-targeting input shifted the rat's choice toward high-benefit, high-cost options in the cost-benefit conflict task but not in the other tasks. By contrast, optogenetic inhibition of the matrix-targeting axons shifted the choice toward high-benefit options except in the cost-cost task. Our results suggest that the PFC-PL corticostriatal pathway is selective for striosomes and controls the animals' choice under motivational conflict, whereas the PFC-ACC corticostriatal pathway is selective for matrix and is involved in the control of motivation toward benefit.

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Title: Properties of striatal beta oscillation at sites identified by microstimulation as controlling approach-avoidance choice behavior

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Abstract: The primate striatum is a node in circuits controlling emotion and motivational states affected in a range of anxiety and obsessive-compulsive disorders. Deep brain stimulation targeting the ventral striatum and related circuits has been applied for the treatment of these disorders, but the neural mechanisms underlying these effects are not fully identified. Here, we adopted a combined approach of focal intrastriatal microstimulation and simultaneous recording of local field potentials (LFPs) in two macaque monkeys performing an approach-avoidance (Ap-Av) decision-making task. The monkeys were required to decide whether to accept or reject

an offered outcome that included both benefit (food) and cost (air puff), whose amounts were indicated in advance by a visual cue. The neural signals in the cue period were dissociated from those related to movements, as the choice was associated with the randomly placed targets. To test for causal evidence of striatal function in such decision-making, we examined the influence of localized microstimulation of the striatum (1-sec trains, biphasic pulses, 70-100 μA at 200 Hz, during cue period) on the Ap-Av choice behavior. At 18 of the 137 sites examined, microstimulation sharply increased avoidance choices (defined as greater than 10% change in the decision boundary), suggesting that the primate striatum has local sites dedicated to modulation of pessimistic states influencing approach-avoidance decisions. We further recorded LFP oscillations from the stimulated sites, performed band-pass filtering (13-28 Hz) to extract beta-band oscillations, and calculated the mean beta-band power during the decision period for each trial. Among 220 channels recorded from the effective sites inducing increased avoidance (allowing overlap in recording from the same sites day to day), beta power during the decision period was positively correlated with the upcoming avoidance decision significantly (Pearson's correlation, $p < 0.05$) on 77 channels (35.0%). At sites at which microstimulation did not induce change in approach-avoidance decision (41 sites), beta power was correlated with the avoidance choice on 16.1% of the recording channels (43/267, including overlap). The frequency of observation of beta power predicting avoidance choice was thus significantly higher in the effective stimulation sites than that in the non-effective sites (Fisher's exact test, $p < 0.01$). These results raise the possibility that pessimistic states can be modulated by local activity in the dorsal striatum, and that striatal local circuits exhibiting beta-band oscillations can participate in such modulation.

Disclosures: K. Amemori: None. **S. Amemori:** None. **D.J. Gibson:** None. **A.M. Graybiel:** None.

Poster

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Canadian institute of health related research (CIHR)

Title: Differential effects of granular and agranular inactivations on a rodent slot machine task

Authors: *P. J. COCKER[1], M. Y. LIN[1], M. M. BARRUS[1], B. LE FOLL[2], C. A. WINSTANLEY[1];
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Abstract: Gambling is a relatively ubiquitous phenomena that many people engage in without adverse effect, yet for a significant minority gambling can become a maladaptive compulsion with phenomenological similarities to substance abuse. Cognitive theories of gambling suggest that the transition from recreational to problematic engagement with gambling is due to the presence of cognitive biases or distortions. One of the most widely recognised of these biases are near-miss effects. Near-misses are unsuccessful outcomes that are structurally proximal to a win. Although subjectively aversive, near-misses have been reliably shown to generate reward expectancy and galvanize further game play. We have developed a rodent slot machine task (rSMT) wherein animals respond to a series of three flashing aperture lights, analogous to the three wheels of a slot machine, nose-poke responses in each hole cause the light to set to on or off. A win is signalled by all three lights setting to on, whereas any other light pattern indicates a loss. At the end of a trial the animal chooses between the 'collect' lever, which delivers 10 sugar pellets on winning trials, but a 10-second time-out penalty on losing trials, or the 'roll' lever which allows the animal to begin a new trial immediately. Animals are sensitive to the number of illuminated apertures presented within the array and 2 out of 3 illuminated apertures are able to generate erroneous responses on the collect lever. Indicating that rodents, like humans, are susceptible to the reinforcing effects of winning signals conveyed within non-winning trials, consistent with a near-miss-like effect. Data from human imaging and lesion studies have implicated an important role for the insula in gambling related decision making. However, imaging studies have yielded relatively inconsistent results and lesion studies lack specificity, such that the relative contributions of insula sub-regions are currently unclear. The granular and agranular insula have differential connectivity and have been ascribed different roles in mediating decision making; a demarcation of function between these two areas may meaningfully inform the neurobiology of disordered gambling. Here, we present data indicating that inactivations of the granular and agranular insula cortex differentially impact reward expectancy on the rSMT. These results have necessarily different mechanistic interpretations regarding the reinforcing effects of near-misses within gambling and consequently different implications for potential treatment options.

Disclosures: P.J. Cocker: None. **M.Y. Lin:** None. **M.M. Barrus:** None. **B. Le Foll:** None. **C.A. Winstanley:** None.

Poster

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Topic: F.03. Motivation and Emotion

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FDV Frontières du vivant

Title: Motivation and reward/effort tradeoff: insights from local field potentials in the ventromedial prefrontal cortex

Authors: *C. VARAZZANI[1], A. SAN-GALLI[1], F. MEYNIEL[2], T. ANDRILLON[3], S. BOURET[1];
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Abstract: One key problem faced by the brain is deciding how much effort to mobilize in order to attain a specific goal. The problem is particularly difficult as it requires not only an initial discounting of potential rewards according to effort costs, but also a persistent monitoring of effort until the goal is reached. The ventromedial prefrontal cortex (vmPFC) has been strongly implicated in representing predictive rewards and choice preferences. What is less clear, however, is the extent to which the vmPFC contributes to discount effort costs from expected rewards. The current study aims at identifying the contribution of the vmPFC to the encoding of such reward/effort trade-off in monkeys. We recorded local field potentials (LFPs) in the vmPFC in two rhesus macaques performing a reward-effort task, where they must squeeze a hand grip (3 levels of effort) in order to get a reward (3 sizes of reward). In each trial, a visual cue indicated both effort level and reward size. The expected value associated to each cue was assessed by measuring the abandon rates (the decision to perform the trial, or not). Abandon rates rose as reward decreased and effort increased, indicating that the reward was discounted by the amount of physical effort required to obtain it. The pupil response, a measure of autonomic arousal, increased with the expected reward at the cue and with the amount of physical effort produced during the action. We used a time frequency decomposition to assess the influence of task parameters on specific frequencies of the LFP signal. Right after the cue onset, the expected reward positively modulated the LFP power ($p<.05$, cluster-corrected) in the 5-30 Hz range. The power in this frequency band was also negatively modulated by the anticipated effort cost ($p<.05$, cluster-corrected). During the action itself, the LFP power in the 5-30 Hz range scaled positively with the imposed effort level (p0.1, $p<.05$). These results suggest that the population activity of vmPFC neurons is affected by the expected value, which depends upon the reward/effort tradeoff. Our results also reveal a relationship between vmPFC and autonomic arousal, as reflected in the positive correlation with pupil dilation. To sum up, the activity of

VMPFC neurons reflects both the expected value prior to the effortful action and the autonomic arousal during its execution.

Disclosures: C. Varazzani: None. **A. San-Galli:** None. **F. Meyniel:** None. **T. Andrillon:** None. **S. Bouret:** None.

Poster

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Title: Individual differences in reward sensitivity modulate ventrolateral prefrontal cortex responses to choice

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Abstract: Human behavioral and neuroimaging studies suggest engaging in choice behavior is inherently rewarding (e.g., Leotti & Delgado, 2011), which leads to enhanced motivation and allocation of cognitive resources towards a particular behavior. Indeed, regions involved in cognitive control, such as the ventrolateral prefrontal cortex (VLPFC), show greater activation during reward-motivated trials (Taylor et al., 2004). One interesting question is whether the opportunity for choice enhances motivation by modulating the engagement of such regions involved in cognitive control. We hypothesized that the opportunity to exert control would engage VLPFC according to individual's sensitivity to rewards. Participants (N = 33) performed a simple decision-making task in an fMRI scanner. On each trial, participants were presented with two options that could either offer similar or dissimilar outcomes. Trials offering similar options effectively forced the participant's choice (i.e., no-choice trials); whereas trials offering dissimilar options allowed the participant to choose freely (i.e., choice trials). Reward sensitivity was measured using the Temporal Experience of Pleasure Scale (TEPS; Gard et al., 2006) and individuals' scores ranged from 26-45. During choice trials (relative to no-choice trials), we found that greater reward sensitivity recruited activity in the VLPFC. Specifically, individuals with greater self-reported reward sensitivity evoked greater VLPFC activation than individuals

with low reward sensitivity. These findings suggest that individuals who are more reward sensitive engage the VLPFC when given the opportunity to choose. These data lend support to the idea that exercising choice enhances motivation via allocating cognitive resources necessary for effective decision making.

Disclosures: **C. Cho:** None. **D.V. Smith:** None. **M.R. Delgado:** None.

Poster

730. Decision Making: Neurocircuitry

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Topic: F.03. Motivation and Emotion

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Title: Neural evidence of good-based economic choice under varying action costs

Authors: ***X. CAI**[1,2], C. PADOA-SCHIOPPA[1];
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Abstract: Previous work showed that economic decisions can be made independently of the spatial configuration of the offers and independently of the action necessary to implement the choice (in goods space). However, goods available for choice may be associated with different action costs. In such conditions, the decision process must necessarily take into account some aspect of the action. Two schemes have been proposed to account for decisions under variable action costs. One possibility is that the brain first computes the "stimulus value" of each option and then combines the stimulus value with the corresponding action cost in an action-based representation. In this scheme, decisions are action-based and take place in premotor regions (Rangel and Hare, 2010). Alternatively, action costs could be integrated with other determinants of value (commodity, quantity, etc.) in an abstract representation. In this view, decisions under variable action costs could take place in the space of goods (Padoa-Schioppa, 2011). To shed light on this fundamental issue, we recorded from the orbitofrontal cortex while monkeys chose between different juices offered in variable amounts and with variable action costs. Specifically, we manipulated the cost associated to each offer by varying the amplitude of the saccade necessary to indicate the chosen option. At the beginning of each trial, the animal fixated the center of a computer monitor. Two offers then appeared on the two sides of the fixation point. Each offer was represented by a set of color symbols. Different colors represented different juices, the number of symbols represented the juice quantity, and the shape of the symbols

represented the saccade amplitude (short or long). Offers remained on the monitor for 1 s, followed by a 1 s delay. Two saccade targets then appeared on the monitor. The color of each saccade target was that of the corresponding juice, the radial distance from the center fixation was set accordingly to the action cost, and the angular position of the two targets was chosen randomly on each trial. After a go signal, the animal indicated its choice with a saccade. Critically, this design provided the opportunity to dissociate in time the decision from the formation of an action plan. We recorded the activity of 754 cells. Different groups of neurons encoded the offer value, the chosen value and the identity of the chosen option. Remarkably, both juice-based and cost-based neuronal representations were present at the same time. Furthermore, chosen juice and chosen cost neurons encoded the choice outcome well before the presentation of saccade targets, indicating that economic decisions were made in goods space.

Disclosures: X. Cai: None. **C. Padoa-Schioppa:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R01NS086104-06A1

Title: Dissociation of local field and action potentials in supplementary eye field during value based decision-making

Authors: X. CHEN[1,2], *V. STUPHORN[1];
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Abstract: Cooling experiments show that neural activity in the supplementary eye field (SEF) causally contributes to value-based decision making. Various decision-related signals have been found in SEF during value based decision tasks. However, SEF occupies an intermediate stage in the neural network underlying decisions. It is therefore not clear, if SEF activity mainly reflects signals reverberating in an extended network, or if any of the signals are computed locally. The cooling experiments disrupted action potentials representing the outcome of local neural computations. Synaptic potentials in dendrites represent input from other brain areas, but they are difficult to record. Local field potentials (LFPs) represent the slower frequency spectrum (below 500 Hz) of extracellularly recorded potentials. The previous literature suggests that both

synaptic inputs and action potentials contribute to LFPs. Thus, the analysis of signals carried by action potentials and LFPs provides a possible way to differentiate between computations within the local cortical network and synaptic input into the network. We therefore recorded LFPs in SEF during a value based decision task, while reversibly blocking action potentials. We had two main results. First, in the cooled cortex, LFP activity in the low-frequency band consistently increased during cooling and likely reflects largely synaptic potentials. In the non-cooled cortex, LFP activity in the high gamma/gamma frequency band was closely correlated with spiking activity. In contrast, during cooling the high gamma/gamma activity was not consistently reduced. In different task periods, activity could be both larger and smaller than in the non-cooled condition. Thus, high frequency LFP activity does not reflect mainly action potentials, but also largely synaptic currents. Second, we found that information about the chosen value, chosen action, and reward evaluation were not only represented in the action potentials, but also in the LFPs. Moreover, these different kinds of information were represented both before and after inactivation. These results suggest that SEF reciprocally receives value and action information from other cortical areas and is part of a larger network contributing collectively to the value based decision process.

Disclosures: X. Chen: None. **V. Stuphorn:** None.

Poster

730. Decision Making: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Title: Free choice in a novel reward preference paradigm: Effects of striatal lesions and diverse control experiences

Authors: *J. RICKER[1], R. KOPCHOCK[2], A. SCHMIDT[2], D. DANIEL[2], A. TYSON[2], H. C. CROMWELL[2];
[1]Bowling Green State Univ., Napoleon, OH; [2]Bowling Green State Univ., Bowling Green, OH

Abstract: Making an advantageous choice between multiple rewards requires an organism to properly and efficiently assign value to each option. Factors such as magnitude of reward, time of delivery, and effort put forth must be taken into account. Reward choice and decision-making have typically been investigated under limited “free” choice. For example, animals often have a choice between one lever and another. This expression of preference is spatially constrained and limited in terms of appetitive actions. The current study employs a novel testing apparatus that

consists of a middle “decision” box, which is connected to two boxes with differing food reward magnitudes. One box is associated with a reward value (1 pellet every 5 seconds), while the other would be associated with another reward value (1.5 pellets every 7.5 seconds). The animals must choose between these two outcome values that combine parameters of magnitude and delay. The total apparatus resembles an expanded conditioned place preference paradigm consisting of 3 boxes and that totals nearly 250 centimeters in length. Our analysis compared free choice among groups: striatal lesion, sham surgery group, no surgery group, and a sucrose-exposed group. This new apparatus also allows for multiple dependent measures to be recorded. These include: food cup checks, total time in box, number of rewards obtained, and ultrasonic vocalizations. These measures allow an examination of free choice in terms of relative reward, reward discrimination, and reward preference. Preliminary data suggests that striatal lesions led to a higher preference for a box with a shorter delay of reward delivery. Our new data investigates differences that may occur between our original striatal lesion groups and groups of “control” rats that have been exposed to different experiences. One “control” group underwent a sham lesion surgery using phosphate buffered saline as opposed to quinolinic acid. A second “control” group had a history of exposure to drinking a sucrose solution. The final “control” group consisted of rats that had no exposure to any surgery or alternate food or water intake. We expect that different experiences will lead to diverse results amongst our control groups. These types of differences between our control groups could allow us to determine how the experience of a sham surgery could alter the overall results of a choice study. This novel design may allow for a fresh perspective on testing reward choice, and it may lead to a better understanding of psychiatric disorders ranging from substance abuse to anxiety disorders.

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Poster

730. Decision Making: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Title: Optical voltage imaging of self-evoked lever-pressing task in head-fixed animals

Authors: C. SONG, *T. KNOPFEL;
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Abstract: Understanding the brain mechanisms underlying behaviour requires identification of neural circuitries involved in the generation of individual behavioural components. Mesoscopic optical imaging of brain activity during these behavioural components using head-fixed animal models expressing optogenetic indicators provides an unparalleled opportunity to achieve this goal. In contrast to mainstream small area two-photon imaging approaches, large area wide-field imaging can capture the integration and distribution of multi-area cortical processing that underlies cognition. Recent advances were made towards understanding the neuronal activity patterns during stimulus-response actions, yet the cortex-wide mechanisms for internally-evoked actions and their differences to stimulus-evoked activity patterns are largely unknown. With this in mind, we developed a lever-pressing task with visual stimulus-associated reward in head-fixed transgenic animals expressing the genetically encoded voltage indicator VSFP Butterfly 1.2 in layer II/III pyramidal cells. We first demonstrated the feasibility of employing a lever-pressing task under stable head-fixation, where the animal is capable of learning and skilfully executing both stimulus-evoked (visual cue) and internally-evoked (self-triggered lever-press) goal-directed reward collection (water licking). Mice were trained to execute these actions while neuronal activities were imaged across large portions of the cortex, including visual, somatosensory and motor cortices.

Disclosures: **C. Song:** None. **T. Knopfel:** None.

Poster

730. Decision Making: Neurocircuitry

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Topic: F.02. Animal Cognition and Behavior

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PICT-2012-1519

Title: Auditory discrimination in a novel spherical treadmill apparatus in head-fixed behaving rats

Authors: A. M. M. MIGUELEZ FERNÁNDEZ[1], A. BURMAN[2], A. I. MARTÍNEZ CÁCERES[2], *B. S. ZANUTTO[3], S. E. LEW[2];
[1]Inst. de Biología y Medicina Exptl. (IBYME-CONICET), Buenos Aires, Argentina; [2]Inst. de Ingeniería Biomédica (IIBM-UBA), Buenos Aires, Argentina; [3]Univ. Buenos Aires-CONICET, Buenos Aires, Argentina

Abstract: While spherical treadmills are widely used in mouse models, there are not many experimental set ups that can be also employed in rats. We introduce a novel training apparatus that allows animals to perform motor responses while being head fixed and can be implemented in lager rodents. The apparatus includes a freely rotating spherical treadmill, an iron structure where animals are head fixed, a fluid delivery system, two video cameras and a software that controls training parameters and performs data storage for posterior analysis. Here we report training two Long Evans rats in an auditory discrimination task. The paradigm employed was of the Go/No-Go type, where walking/not-walking on the treadmill after presentation of an auditory stimulus was rewarded with a drop of water depending on the frequency of the stimulus. Rats showed a favorable response to the training apparatus, handling and procedures and were able to successfully discriminate between a 8KHz and a 1KHz stimulus and execute the correct response.

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Poster

731. Motivation and Emotion: Reward III

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Topic: F.03. Motivation and Emotion

Support: Italian Ministry of Health "Ricerca Corrente 2014".

Title: Prelimbic α1-adrenergic receptors modulate extinction of amphetamine-induced conditioned place preference

Authors: *S. PUGLISI-ALLEGRA[1,2], P. SACCOCCIO[1], C. MILIA[1], P. CAMPUS[1], E. LATAGLIATA[2];
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Abstract: Norepinephrine (NE) in medial prefrontal cortex (mpFC) is critical for the acquisition of conditioned place preference (CPP) based on different addictive drugs or natural rewards (Ventura et al., 2003 J Neurosci, 23(5): 1879-1885; Ventura et al., 2007 Proc Natl Acad Sci U S A. 104 (12):5181-5186). Moreover, it has been demonstrated the involvement of prefrontal NE also in the modulation of the extinction of conditioned response, in particular, through its action on β-adrenergic receptors (LaLumiere et al., 2010 Learn Mem 17, 168-175. ; Otis et al., 2013 J

Neurosci 33, 1271a-1281a). However, recent results have suggested a potential contribution on the extinction of appetitive conditioned response also of α1-adrenergic receptors (Bernardi and Lattal 2010 Behav Neurosci Apr;124(2):204-; Bernardi and Lattal Neuroreport. Dec 19;23(18):1048-51.; 2012), although their role in mpFC has not yet been fully clarified. Here, we assessed whether and how α1-adrenoceptors in the prelimbic (PL) portion of the mpFC are involved in modulation of the persistence of drug-associated cue memories. We investigated the effects of the α1-adrenoceptor antagonist Prazosin infusion in PL mpFC on extinction of acquired CPP to amphetamine and on expression of c-Fos levels in Nucleus Accumbens (NAc) core and shell of C57BL/6J mice in a spontaneous extinction paradigm (subsequent daily testing sessions). In Prazosin treated mice the place preference is no more evident from the first day of extinction, while in the Vehicle group the preference persists for further thirteen days. Moreover, Prazosin treated mice showed a lower c-Fos expression in both NAc core and shell region in comparison with Vehicle group. These results indicate that prefrontal cortical NE contributes to delay extinction of memories associated with drugs, modulating NAc activity, through α1-adrenergic receptors in PL cortex.

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Poster

731. Motivation and Emotion: Reward III

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Title: Outcome monitoring and behavioral adjustment by putative pyramidal neurons and interneurons in the primate anterior cingulate cortex during a reversal learning task

Authors: *T. KAWAI[1], H. YAMADA[1], N. SATO[2], M. TAKADA[3], M. MATSUMOTO[1]; [1]Fac. of Med., Univ. of Tsukuba, Tsukuba, Japan; [2]Dept. of Psychological Sci., Kwansei Gakuin Univ., Nishinomiya, Japan; [3]Primate Res. Institute, Kyoto Univ., Inuyama, Japan

Abstract: The anterior cingulate cortex (ACC) is known for its crucial roles in monitoring the outcome of a choice and adjusting a subsequent choice behavior. In the present study, we investigated how different types of ACC neurons, i.e., pyramidal neurons and interneurons, contribute to these two processes. We trained two monkeys to perform a reversal learning task. While the monkey was gazing a fixation point, two saccadic targets were presented on both the left and the right sides of the point. The monkey was required to choose one of the targets with a saccade. Choosing one target was followed by a liquid reward with 50% probability, whereas choosing the other was followed by no reward. The reward-position contingency was fixed within a block of 20 to 40 trials, and then reversed without any instruction. The monkey adjusted their choice behavior based on past outcome experiences. We recorded the activity of 329 ACC neurons. Depending on their spike waveforms, 227 neurons were classified as putative pyramidal neurons and 102 were classified as putative inhibitory interneurons. We found that both types of neurons transmitted signals that were associated with outcome monitoring and behavioral adjustment. Indeed, 153 of the 227 pyramidal neurons (67%) and 71 of the 102 interneurons (70%) exhibited a significant outcome-dependent modulation of their activity (i.e., a significant difference between their reward and no-reward evoked responses) ($p < 0.05$, Wilcoxon rank-sum test). On the other hand, 34 of the pyramidal neurons (15%) and 13 of the interneurons (13%) showed a significant adjustment-dependent modulation that predicted whether the monkey would shift the current choice to the alternative in the next trial ($p < 0.05$, Wilcoxon rank-sum test). There were no significant differences in the proportion between the pyramidal neurons and the interneurons ($p > 0.05$, Fisher's exact test). However, a notable difference between the neuron types was seen in the magnitude of the outcome-dependent modulation. We divided the recorded neurons into two subgroups; neurons more strongly activated by no reward than the reward were classified as "negative-outcome type" and those more strongly activated by the reward than no reward were classified as "positive-outcome type". In the negative-outcome type, interneurons exhibited a stronger outcome modulation than pyramidal neurons. In the positive-outcome type, on the other hand, pyramidal neurons showed a stronger outcome modulation than interneurons. These data suggest that pyramidal neurons and interneurons in the ACC may participate in outcome monitoring in distinct ways.

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Poster

731. Motivation and Emotion: Reward III

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Topic: F.03. Motivation and Emotion

Support: Klingenstein foundation

Title: Reward simulation in orbitofrontal cortex

Authors: *Z. WANG, B. Y. HAYDEN;
Univ. of Rochester, Rochester, NY

Abstract: Evaluation is critical to reward-based decisions. A key question about the evaluation process is: While evaluating each offers (reward stimulus), how does neural machinery recollect previous reward experience with the offer and encode the value associated with the experience? We hypothesized that the decision-making circuits achieve this evaluation process via reward simulation. We defined reward simulation as representing reward stimulus by reactivating neural activity patterns that are evoked by actual receipt (experience) of a reward. Previous studies have shown that orbitofrontal cortex (OFC) is involved in reward representation and value encoding through credit assignment to stimulus. Here, we used a novel task paradigm and single-unit recording in rhesus macaques to record from neurons in Area 13 of OFC. We wanted to know how neurons encoded information about reward when it was offered (and thus motivated choice) and when it was received (and thus did not motivate choice). We also wanted to know how encoding of reward changed across context when the reward was experienced vs. merely described. To answer these questions, we compared coding format of offer and reward within context and experienced vs. described offers across contexts. We defined coding format as the set of regression weights from regression of firing rates against reward magnitude. We find that offer and reward were encoded using the same format within context. And yet, across context, experienced and described offers were not coded with the same format. These results suggest a common reward simulation process in both experienced and described contexts but different coding scheme of offers across context.

Disclosures: Z. Wang: None. **B.Y. Hayden:** None.

Poster

731. Motivation and Emotion: Reward III

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Program#/Poster#: 731.04/CC37

Topic: F.03. Motivation and Emotion

Title: Formation of a pair bond occurs during the nest coo phase of the breeding cycle in ring doves, Streptopelia risoria

Authors: A. M. DIOS[1], *M.-F. CHENG[2];
[1]Psychology, [2]Rutgers Univ., Newark, NJ

Abstract: Pair bonding is typically measured by an animal maintaining a preference for its mate. In ring doves, males that have gone through a breeding cycle will seek out their mate in a large aviary and will prefer to spend time with their mate despite a seven month period of separation (Morris and Erickson 1971). Our current study sought to determine whether pair bonding occurs prior to the completion of a breeding cycle. Our laboratory has found that a neuronal marker for pair bonding, ZENK counts in the nucleus taeniae, is more accurate than preference tests at predicting whether doves have formed a pair bond (Dios et al 2013). Using this marker, we found that both male and female doves form pair bonds during the courtship (nest coo) phase of the breeding cycle. In female ring doves, the nest coo phase of the breeding cycle triggers the hypothalamic-pituitary ovarian system which culminates in ovulation (Cheng et al 1998; Cheng and Balthazart 1982). The formation of a bond at this stage of the breeding cycle, suggests that males and females may be predicting the successful completion of a breeding cycle with their mates- a concept in line with the evolutionary view that pair bonding is a reproductive strategy used to ensure survival of the individual forming the bond.

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Poster

731. Motivation and Emotion: Reward III

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 731.05/CC38

Topic: F.03. Motivation and Emotion

Support: UNAM DGAPA IN224214

FESI PAPCA 2014-54

Title: The effect of estrous cycle on binge-eating behavior induced by intermittent access to sucrose in rats

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Abstract: Binge Eating Disorder (BED) is characterized by episodes of consumption of an amount of food that is significantly larger than most individuals would eat under similar circumstances in a discrete period of time. It is well known that the prevalence of BED is higher among women than among men and that high-carbohydrates foods are preferred during a binge eating episode. Since the hormone estradiol has the ability to modulate the food intake and body weigh it has been suggested that the cycling concentration of female hormones through the estrous cycle may inhibit food intake during binge eating. Accordingly, the aim of our study was to evaluate the effect of estrous cycle on binge-eating behavior of sucrose in adult rats. Independent groups of Sprague Dawley female rats (250-350 g) were individually housed and its estrous cycle phase was determined by the relative amount of cells in vaginal smears dyed with cresyl violet and examined under light microscopy. The subjects were matched by weight in three different groups and were differentially exposed to a sucrose solution (10% w/v): no access (control), intermittent access (2 h a day), and ad libitum access (24 h per day) throughout 28 days with standard chow and tap water ad libitum. All animals started the BED-inducing protocol at the beginning of diestrous phase, food and sucrose solution intake (2 and 24 h access groups) and estrous phase were measured on a daily basis. As expected, we found that the sucrose intake of intermittent access group significantly increased (compared to 24 h access group) during the final 5 days (24-28) of the protocol; however this effect was independent of the estrous phase, since the intake of chow, sucrose or total intake (sucrose+chow) was not different between groups in estrous or diestrous phase. When data were split by initial days of training and days of full established binge-eating behavior, we did not find any difference on sucrose intake between rats in estrous or diestrous phase. Our findings suggest that the inhibitory effect of estrous phase on the food intake did not affect the establishment of binge-eating induced by intermittent access to a sucrose solution.

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Poster

731. Motivation and Emotion: Reward III

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Topic: F.03. Motivation and Emotion

Support: NSERC

Title: Prosexual effects of a cabergoline derivative with 5-HT 2b antagonist binding properties

Authors: *R. A. ANTONIE, J. PFAUS;
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Abstract: Cabergoline is a unique ergot derivative typically prescribed off-label to treat low sexual desire and anorgasmia associated with hyperprolactinemia and similar effects of chronic SSRI medication. Compound Number 9 (CN9) is a semi-synthetic proprietary cabergoline derivative with a similar pharmacological profile, but with 5-HT2b antagonist, instead of agonist, properties. This makes CN9 a potential alternative to cabergoline as it is unlikely to induce the cardiac fibrosis that comes with high-dose systemic administration. The objective of the current study was to assess whether chronic administration of CN9 retains the prosexual effects of cabergoline. Male Long-Evans rats (N=40) were given 5 multiejaculatory sexual experiences at 4-day intervals in bilevel chambers prior to daily oral administration by gavage of one of three doses of CN9 (0.003, 0.015, or 0.03 mg/ml/kg) or the saline control for 36 days. During this period, males received an additional 9 copulation tests in bilevel chambers at 4-day intervals. Similar to cabergoline, the medium and high doses of CN9 produced a dramatic and significant increase in ejaculations and a decrease in the post-ejaculatory interval compared with the low dose and control. These effects were observed at the beginning of test number 3 and persisted throughout the treatment regimen, suggesting a lack of tolerance to CN9. These results demonstrate that CN9 has prosexual effects in sexually experienced males, suggesting that it can be a viable alternative to cabergoline. To examine the pattern of brain activation by CN9, rats were gavaged with their corresponding dose of the compound or control 4 days following the last copulatory trial and sacrificed for immunohistochemical analysis of Fos protein. The number of Fos positive cells in the nucleus accumbens, medial preoptic area, and ventromedial hypothalamus of rats given CN9 are being examined. Funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

Disclosures: R.A. Antonie: None. **J. Pfaus:** None.

Poster

731. Motivation and Emotion: Reward III

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Topic: F.03. Motivation and Emotion

Support: CONACYT 129337

Title: Effects of sucrose concentration on performance on a progressive schedule in rats

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Abstract: Rats prefer sucrose over chow and their palatability effect produces high breakpoints (BK) in a progressive ratio schedule (PR). The progressive ratio schedule has been utilized as a procedure to estimate the reinforce value of a substance and the motivational state of an organism. While in a fixed ratio (FR) schedule the requirement for the reinforce delivery is constant throughout a session, in a PR the number of responses required for the delivery of reinforcer increases constantly. The BK has been considered as both a measure of the motivational state and of the incentive value of the reinforcer; however, in several studies has been demonstrated that the BK is sensible to non-motivational procedures. Killen and Bradshaw have made an adaptation of the Mathematical Principles of Reinforcement to apply such model to PR schedules. According to MPR, the performance on PR schedules may be determined by three parameters; one of them is a parameter of specific activation, a, that estimates the organism's motivational state sensitive to level of the food deprivation. The aim of this work was to determine the effect of the changes in motor and motivational parameters associated to the use of different sucrose concentrations. Method: Twelve Male Wistar rats were evaluated into the PR for four concentrations of sucrose (0.178, 0.31, 0.56 & 1M) daily until they got stabilization criteria (les than 10% variation in BK) in 45 sessions approximately. Higher response rate was observed with the 0.56M concentration and the lower response rate with the 0.178M; although such differences in response rate did not attain statistical significance, performance was well described by fitting the model to performance data.

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Poster

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Topic: F.03. Motivation and Emotion

Support: P50 AA017072

Title: Nucleus accumbens shell orexin signaling promotes alcohol drinking in mice

Authors: *K. LEI, F. W. HOPF, S. A. WEGNER;
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Abstract: Multiple neural systems are involved in the regulation of alcohol consumption. Previous research has demonstrated that orexin (OX) signaling, particularly via the type-1 receptor (OX1R), promotes alcohol drinking. Here, we used C57BL/6 mice to elucidate the role of OX1R signaling in alcohol consumption in a 2 bottle choice drinking paradigm. Adult, male, mice were allowed to drink a 15% ethanol solution or water for 2-hr/day, starting 3-hr into the dark cycle. We first systemically administered various doses (0, 0.3, 1, 3, or 10-mg/kg) of SB-334867 (SB), an OX1R selective antagonist, and found that global blockade of OX1R reduced alcohol consumption in a dose-dependent manner, with alcohol consumption significantly reduced only by 10-mg/kg SB. We then microinfused SB into selected areas of the brain to determine the region(s) in which OX1R signaling acts to promote alcohol drinking. The nucleus accumbens shell (NAsh) was found to be one region where OX1R blockade reduced alcohol intake in mice. SB within the NAsh also reduced operant alcohol intake in Wistar rats. In addition, there was a positive correlation, in mice, between the effect of NAsh SB on alcohol drinking and basal alcohol intake. We also performed *in vitro* electrophysiology experiments in adult, alcohol-drinking mice, and found that orexinA (100 nM) increased action potential firing in NAsh neurons, with no effect on input resistance or evoked AMPA receptor currents. Furthermore, pretreatment with SB blocked the orexinA mediated increase in cell firing. Together, our data suggest that Ox1Rs act within the NAsh to promote alcohol drinking, with a correlation between drug efficacy and the amount of alcohol consumed. We are currently investigating other brain regions that may be involved with alcohol consumption regulation through OXR signaling. This research is supported by P50 AA017072.

Disclosures: K. Lei: None. **F.W. Hopf:** None. **S.A. Wegner:** None.

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Title: Compulsion-like alcohol drinking changes ampars and d-serine-inhibited nmdars in the accumbens core

Authors: T. SEIF[1], J. A. SIMMS[2], K. LEI[2], S. WEGNER[2], A. BONCI[4,5], R. O. MESSING[6], *F. W. HOPF[3,2];
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Abstract: Compulsive drinking despite adverse social, legal, and physical consequences is a central hallmark of human alcohol use disorders (AUDs) and is a pernicious impediment to treatment. The specific brain circuits that promote this compulsive behavior are poorly understood. We have shown in rats that connections from the anterior insula and medial prefrontal cortex (mPFC) to the nucleus accumbens core (NACore) are critical for driving alcohol intake in a model of compulsion-like alcohol drinking (CLAD), where rats drink alcohol despite adulteration with quinine or despite the presence of footshocks. This compulsion-like drinking also requires activation of hyperpolarization-active NMDARs (HA-NMDARs) within the NACore (Seif et al., 2013, Nat Neurosci 16:1094). We recently demonstrated that the NMDAR modulator D-serine selectively suppresses CLAD when infused within the NACore or systemically; we also found that D-serine inhibits mPFC-evoked HA-NMDAR activity in the NACore, as measured with *in vitro* electrophysiology and optogenetics (Seif et al., 2015, NPP epub). This study involved Intermittent Alcohol Access (IAA, 20% alcohol under 2-bottle choice, 3 overnights starting Mon., Wedn. and Frid. afternoons). After ~2.5 mo IAA, rats then drank alcohol 20-min/day, 5-day/wk for ~2 mo before *in vitro* analysis of HA-NMDARs. Here, we show that mPFC-evoked, D-serine-inhibited HA-NMDARs also appeared after 4-5 mo IAA, together suggesting that NACore HA-NMDARs appear after different patterns of alcohol drinking, in addition to promoting CLAD. Thus, D-serine may represent an FDA-approved, immediately accessible pharmacotherapy for treatment of compulsive aspects of human AUDs. We also combined *in vitro* electrophysiology and optogenetics to show functional adaptations in

cortically-evoked AMPARs in the NACore from alcohol-drinking rats, in addition to HA-NMDAR changes. In alcohol-naïve rats, the AMPAR-NMDAR ratio at +40 mV was lower in aINS-NACore inputs relative to mPFC-NACore inputs or electrically-evoked NACore inputs. In alcohol-drinking rats, the AMPAR-NMDAR ratio was decreased in both aINS-NACore and mPFC-NACore but not electrically-evoked inputs, relative to alcohol-naive. AMPAR EPSC kinetics were similar for all conditions (ChR2-evoked vs electrical, naïve vs alcohol drinker), except for a slowed AMPAR decay in aINS-NACore inputs in drinkers. Thus, long-term alcohol intake alters both HA-NMDARs and AMPARs under aINS and mPFC cortical inputs to the NACore. The behavioral impacts of AMPAR changes in the NACore, and possible interactions of cortically-activated HA-NMDARs and AMPARs, remain to be elucidated.

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Poster

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Title: Sign-tracking rats have more thalamic mast cells than goal-tracking rats

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Abstract: Mast cells contain numerous neurotransmitters within their granules (e.g., histamine, serotonin, and cytokines), and are capable of influencing neuronal activity. Degranulation of mast cells occurs in response to a variety of behavioral states and drugs of abuse, and mast cell activity influences behavior even under basal conditions. However, it is unknown whether mast cells in the brain contribute to addiction vulnerability. Attribution of incentive motivational properties to reward-related cues is believed to be a key etiologic factor underlying addictive behavior, particularly relapse. Individual differences in the attribution of incentive salience can be measured using a Pavlovian conditioned approach (PCA) procedure, wherein some rats,

termed sign-trackers (STs), attribute incentive salience to reward cues and are more vulnerable to addiction-like behaviors while other rats, termed goal-trackers (GTs) and intermediate responders (IRs), do not. STs have been shown to have higher neural activity within thalamic brain regions in response to both food- and drug-related cues, and brain mast cells are selectively concentrated in the thalamus. Using a PCA procedure, we screened rats over the course of seven daily PCA training sessions, waited a week to restore baseline activity, then quantified thalamic mast cells using a toluidine blue stain. We found that STs, compared to GTs and IRs, have higher numbers of mast cells within the thalamus. These results suggest that thalamic mast cell signaling may contribute to individual differences in the attribution of incentive salience to reward cues and, therefore, addiction vulnerability.

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Poster

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Title: Orbitofrontal cortex mediates inhibition within the basolateral amygdala to promote appetitive Pavlovian conditioning

Authors: *B. T. SAUNDERS[1], K. R. VITALE[2], P. H. JANAK[1];
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Abstract: Pavlovian conditioning is mediated in part by the strengthening of excitatory synapses onto basolateral amygdala (BLA) neurons that carry sensory information about the conditioned cue. Many single-unit recording studies find that a subset of BLA neurons are excited by a conditioned cue after either appetitive or aversive Pavlovian conditioning, which is thought to mediate behavioral responding. To further explore how the amygdala contributes to this process, we recorded single-unit activity in the BLA of rats as they underwent appetitive Pavlovian conditioning. Training consisted of repeated presentations of a 30-s auditory stimulus (CS+) paired with sucrose solution delivered at a variable latency. An equal number of presentations of a different sound (CS-) are paired with nothing. We found that more than half of recorded

pyramidal neurons were inhibited by the CS+, while only <10% were excited. BLA inhibition was highly correlated with behavior during acquisition, expression, and extinction task phases. We next asked whether this inhibition is required for the expression of conditioned cue responding, via bilateral optogenetic activation of ChR2-transduced BLA neurons. On a test session after training on the same task, we activated the BLA with blue laser (10s@20Hz) on 50% of CS+ trials. Rats showed reduced conditioned port entry on stimulated versus unstimulated trials. As the BLA is a complex structure that contributes to both aversive and appetitive behavior, one possible role for cue-evoked inhibition is to suppress competing behaviors and facilitate decision making during Pavlovian responding. To investigate this possibility, we examined the effect of pharmacological inactivation of the orbitofrontal cortex (OFC), an area involved in decision making. OFC inactivation reduced cue-evoked BLA inhibitions and impaired conditioned port entry behavior. We next looked at the role of OFC inputs directly on behavior by transducing OFC neurons with a viral vector containing the inhibitory opsin ArchT3.0 and targeting optic fibers to the BLA. On test day, we inhibited OFC terminals in the BLA with green laser (10s pulse) on 50% of CS+ trials. Rats spent significantly less time in the port on stimulated trials compared to unstimulated trials, indicating that direct input from the OFC to the BLA mediates conditioned responding. These data suggest a critical role for BLA inhibition and an OFC-BLA circuit in appetitive Pavlovian conditioning, and expand our understanding of how cortico-amygdalar interactions contribute to motivated behavior. We are currently exploring local circuit connectivity to understand how BLA inhibitions are generated.

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Topic: F.03. Motivation and Emotion

Support: Emerging Field Initiative Neurotrition FAU Erlangen-Nuremberg

Title: The effect of potato chip craving on the human brain: an fMRI study

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[1]Inst. For Pharmacol. and Toxicology, Erlangen, Germany; [2]Child and Adolescent Psychiatry, Erlangen, Germany; [3]Food Chem. Unit, Dept. of Chem. and Pharm., Erlangen, Germany

Abstract: Hedonic hyperphagia - eating for pleasure independent from hunger - is a phenomenon nearly everybody knows. Snack food like potato chips are ingested without homeostatic need and may lead to non homeostatic energy intake. Our aim was to detect modulations in neural activity due to chips craving in humans. For this purpose, we used standard 3T BOLD fMRI to investigate brain activation patterns in 15 humans (8 female, 7 male, age 24-45, BMI 23,6 ± 3.1) induced by visual application of either chips or non hedonic food such as zucchini and cucumbers. Before each fMRI session the participants were informed that they were allowed to eat either chips or zucchini for 2 minutes after the fMRI session. Each participant was measured two times, eating chips after the first and zucchini after the second session. The obtained BOLD fMRI activation patterns were correlated with individual "hedonic scores" determined from a questionnaire that was designed to evaluate general food craving behavior. Additional to this general craving behavior the participants were asked for their current appetite to chips or zucchini. The questionnaire had to be answered before and after the experimental session. As expected, the usual affinity to chips was much higher than to zucchini and consequently the percentual amount of eaten chips was also higher. Interestingly, eating chips seems to enhance the craving for chips whereas the "craving" for zucchini remained unchanged after eating zucchini. The activation of basal ganglia (predominantly striatum and nucleus accumbens), insula and precuneus were strongly modulated due to visual stimulation with chips. The activated volume of the striatum was significantly enhanced; its maximum BOLD response amplitude (activation intensity) was significantly reduced in both sessions. Instead the activity of the nucleus accumbens, an important structure of the reward system, was only modulated during the first session when the participants expected to eat chips after the session. The insula also showed different activation changes depending on the provided food. Expecting chips seems to be related to an increasing activation volume due to visual stimulation with chips whereas during the zucchini-session the insula's activated volume was decreased. Additionally, BOLD activation of striatum and precuneus, activation volume as well as activation intensity, was strongly negative correlated to the individual hedonic score. In conclusion, visualizing potato chips in the context of expected food influence the activity of several brain structures responsible for reward, motor control and self-awareness.

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Poster

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Title: The striatum multiplexes distinct reward signals

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Abstract: A wide range of rewards -- from monetary incentives to praise from a supervisor -- shape our behavior and contribute to the substantial variability observed across the population. To study the neural underpinnings of reward processing, researchers have employed various paradigms that isolate responses associated with the receipt of reward. In one popular paradigm, participants are presented with a card and asked to guess whether the number on the card is higher or lower than the number five (Delgado et al., 2000). When participants guess correctly, they gain money; but when participants guess incorrectly, they lose money. Comparing responses to gains relative to losses has revealed widespread activation across the striatum in multiple populations, including healthy adults (e.g., Hariri et al., 2006), adolescents (Forbes et al., 2009), and older adults (Cox et al., 2008). Yet, these canonical observations may obscure other, unidentified relationships between reward and the striatum. Indeed, the striatum is composed of multiple interacting functional subunits that are difficult to examine using standard neuroimaging resolution (e.g., 3.5 cubic mm voxels) and analytical approaches that do not take into consideration how distinct spatiotemporal responses. To investigate this issue, we measured striatal responses using high-resolution neuroimaging (1.8 cubic mm voxels) in participants (N = 22) playing the card task. We quantified the spatiotemporal responses to monetary gains and losses using tensorial independent component analyses (Beckmann & Smith, 2005) and dual-regression analysis (Smith et al., 2014). Consistent with prior work using the card task, we found one spatiotemporal component within the striatum that exhibited an increased response to gains (relative to losses). Strikingly, we also observed another spatiotemporal component within the striatum that exhibited increased responses to losses (relative to gains). Our findings demonstrate that the striatum multiplexes distinct reward signals: monetary gains and losses are simultaneously represented by distinct spatiotemporal response patterns within the striatum. These observations highlight how advanced analytical approaches in neuroimaging can enhance knowledge about the spatiotemporal contributions of regions such as the striatum to reward processing.

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Poster

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Topic: F.03. Motivation and Emotion

Title: Neural value estimations related to limited partner perspective during social exchange

Authors: *A. VALDESPINO[1,2], B. T. HILTON[2], H. SULLIVAN-TOOLE[2], B. KING-CASAS[2], J. A. RICHEY[2];
[1]Psychology, [2]Virginia Tech., Blacksburg, VA

Abstract: The current study leveraged neuroeconomic techniques during fMRI to probe whether brain regions supporting value computations assign positive value to partner uncertainty in a social exchange. SUMMARY Introduction: The vmPFC has been implicated in computing abstract social rewards such as being liked, or understood (Davey et al., 2010; Morelli, Torre & Eisenberger 2014). We leveraged a social neuroeconomic task in which it is inherently valuable, from participants' perspectives, for an interaction partner to be uncertain about participants' financial resources. Specifically participants could exploit partners' limited perspectives and keep more money for themselves. We hypothesized that regions supporting abstract reward computations (vmPFC) would positively scale with partner uncertainty. Consistent with hypotheses, reward-computing subregions of vmPFC positively scaled with partner uncertainty. Method: Students at Virginia Tech (N=20; mean age = 19.9, SD = 1.6) completed a One-Sided Uncertainty Ultimatum Game (Rapoport & Sundali, 1996). For each round, participants offered a split of a financial 'pie' between themselves and a responder. Responders did not always know the pie-size with certainty and uncertainty was systematically varied. Specifically, under conditions of certainty, responders knew the pie size; under conditions of uncertainty, responders knew the pie would be drawn from a numeric range, without knowing the exact pie. Uncertainty was manipulated by increasing the range by increments of $10 across runs. Analyses (preprocessing, first- and second-level) were conducted in FSL (Smith et al., 2004). To assess brain activity scaling with partner uncertainty, activity during the decision phase was orthogonalized with respect to trial-wise money kept, offered, and percentage money offered. We performed a linear trend analysis on the orthogonalized decision phase activity by weighting scan run [certainty, low uncertainty, medium uncertainty, high uncertainty] as [0,1,2,3], respectively. Results: Behaviorally, there was a significant main effect of certainty, indicating proposers offered participants less as uncertainty increased ($p<.001$). Neurally, orthogonalizing relevant aspects of the design including money kept and given, activity within a cluster spanning the vmPFC/mOFC scaled monotonically with increasing partner uncertainty ($p<.01$, FDR corrected). Discussion: Brain regions implicated in reward-computations scaled positively with partner uncertainty, possibly indicating these regions assign a value tracking the amount of information interaction partners reveal to each other.

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Topic: F.03. Motivation and Emotion

Title: Roles of the medial prefrontal cortex and basolateral amygdala in punished ethanol seeking

Authors: *A. KOCHARIAN, L. R. HALLADAY, A. HOLMES;
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Abstract: Compulsive ethanol-seeking despite aversive consequences is prevalent among those with alcohol use disorders (AUD). Evidence has shown that circuitry within the basolateral amygdala (BLA) and the medial prefrontal cortex (mPFC) modulates the persistence of drug-seeking, and may contribute to the suppression of behavior during punishment. For example, shock-induced suppression of cocaine-seeking is attenuated by lesions to the BLA (Pelloux et al., 2013). Additionally, while the prelimbic region of mPFC (PL) has been proposed to promote drug-seeking behaviors (Corbit, 2003), the infralimbic region (IL) may function to inhibit drug-seeking in a variety of behavioral settings, such as following extinction training (Peters, et al., 2008). Prefrontal cortical control over limbic-striatal systems has been thought to drive executive top-down control over drug seeking, and impairments in these circuits might underlie the persistence of drug seeking in the face of adverse outcomes. *In vivo* recording studies conducted in our lab (unpublished data) have demonstrated that neurons in BLA and IL are responsive to ethanol-seeking in a manner that not only depends upon whether animals have received punishment or not, but also upon whether individual mice are resistant or sensitive to punishment. To further examine the role of BLA and IL in punished reward seeking, we employed an operant task in which C57BL/6J mice were first trained to reliably lever-press for (10%) ethanol, and then given a punished probe test during which every other lever-press produced a footshock. Punishment consisted of a 40 minute operant session, during which 50% of lever presses were followed by a 0.75 second, 0.3 mA footshock. Retention of punished-suppression of ethanol-seeking was measured on each of the next two days, via a 40-minute unpunished, rewarded session (FR1). We used an optogenetic approach to silence (with the inhibitory opsin, archearhodopsin) neurons in either BLA or IL during the retention test,

specifically during periods when the mouse entered a designated “reward lever zone”. These findings could provide novel insight into the circuitry modulating compulsive responding for ethanol. Research funded by the NIAAA Intramural Research Program.

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Support: Davis Foundation Fellowship on Eating Disorder Research

Title: Application of neural ensemble labeling techniques to the investigation of aversion encoding in the ventral striatum

Authors: *D. M. OPLAND, C. W. BOND, D. S. ABRAMOV, D. OTTENHEIMER, R. J. DILEONE;
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Abstract: The ventral striatum is known to take part in responses to both primary appetitive and aversive stimuli, a component of its role in mesocorticolimbic reward processing. Previous research has elucidated the role of D1R-containing medium spiny neurons (MSNs) in encoding appetitive stimuli and potentiating behavior (“Go” neurons). Conversely, D2R-MSNs encode aversive stimuli and attenuate behavior (“No-Go” neurons). Recent evidence suggests that both D1R- and D2R-MSNs are activated by aversive stimuli. It has yet to be determined whether these subpopulations encode similar information (cooperative encoding schema) or opposing information (competitive encoding) where behavioral output is dependent on output of the dominant subpopulation. We have applied a molecular technique that allows for labeling and optogenetic manipulation of whole neural ensembles to investigate aversive stimuli encoding in the ventral striatum (nucleus accumbens medial core/shell boundary). This technique utilizes a transgenic mouse line (ArcTRAP) in which tamoxifen-inducible Cre recombinase is driven by the endogenous Arc promoter permitting labeling of neural ensembles active during an 8-hour time window. In our experiment neural ensembles activated by exposure to an aversive stimulus (5x 1s, 1mA foot shocks) are labeled following viral-mediated microinjection of Cre-inducible channelrhodopsin-eYFP to the ventral striatum. Subsequent optogenetic reactivation of these ensembles during the conditioning epochs of a conditioned place preference (CPP) paradigm was

sufficient to produce place aversion without locomotor effects suggesting aversive encoding was captured. In addition to whole ensemble labeling experiments we are developing a novel molecular tool, the 2-key FLEX switch, in which stable expression of the target gene (channelrhodopsin-eYFP) is dependent on both Cre and Flp recombinase activity. This utilizes an AAV-vector where a synthetic Arc promoter drives expression of tamoxifen-inducible Flp recombinase, yielding activity dependence and temporal constraints as in the ArcTRAP mouse. Combining the 2-key FLEX switch with the Arc-Flp virus in D1R-Cre and D2R-Cre transgenic mice allows labeling of just D1R- or D2R-MSNs activated by footshock. This system can be used to interrogate the encoding of aversion in the two major populations of ventral striatal projection neurons.

Disclosures: D.M. Opland: None. **C.W. Bond:** None. **D.S. Abramov:** None. **D. Ottenheimer:** None. **R.J. DiLeone:** None.

Poster

731. Motivation and Emotion: Reward III

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 731.17/CC50

Topic: F.03. Motivation and Emotion

Support: Colorado Brain Trust

Title: Surface-based morphometry in lateral prefrontal cortex is associated with reward processing and impulse inhibition in combat deployed veterans with post-traumatic stress disorder

Authors: *N. D. FOGLEMAN, F. NAAZ, B. E. DEPUE;
Dept. of Psychological & Brain Sci., Univ. of Louisville, Louisville, KY

Abstract: A significant portion of previously deployed combat Veterans from Operation Enduring Freedom and Operation Iraqi Freedom/Operation New Dawn (OEF/OIF/OND) are affected by post-traumatic stress disorder (PTSD). Despite this fact, neuroimaging studies examining whether the neural correlates of cognitive control, outside of emotion provocation paradigms, are compromised within this population are extremely sparse. Our previous work suggests that individuals with PTSD have difficulties with inhibitory control (as assessed by a response inhibition task) and that these difficulties are associated with volumetric differences in limbic regions. The current study further examined whether disinhibited behavior was exhibited when these individuals performed a task involving reward processing. Using the modified Iowa

Gambling Task (mIGT), we investigated whether differences in surface-based morphometry (SBM), assessing cortical volume, thickness and area, were associated with behavioral measures related to reward processing and impulse inhibition. Results indicated abnormal morphometry in specific regions of the lateral prefrontal cortex (LPFC), including the orbitofrontal cortex (OFC) predicted poorer performance on the mIGT. Furthermore, morphometric results were related to an individuals' level of symptomatology. The lateral prefrontal cortex (LPFC), known for its role in cognitive control, and orbitofrontal cortex (OFC), an area involved in making stimulus-reward associations with the reinforcement of behavior appear to be compromised in this population. Furthermore, these findings suggest that morphometric changes in the OFC in OEF/OIF/OND Veterans with PTSD are associated with cognitive and cognitive-emotion integration deficits in reward processing and impulse inhibition.

Disclosures: **N.D. Fogleman:** None. **F. Naaz:** None. **B.E. Depue:** None.

Poster

731. Motivation and Emotion: Reward III

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 731.18/CC51

Topic: F.03. Motivation and Emotion

Title: Effects of attentional context and stimulus valence on cortical-limbic responses in youth

Authors: *R. MANIMALETHU[1], T. BAWA[1], K. RAMASESHAN[1], M. RE[2], P. BRAMBILLA[3], V. DIWADKAR[1];
[1]Wayne State Univ., Detroit, MI; [2]Univ. of Udine, Udine, Italy; [3]Univ. of Milan, Milano, Italy

Abstract: Background: Attentional context and stimulus valence are strong modulators of cortical activity. Using a continuous performance task (Soloff et al., 2015), we manipulated congruence between context and valence and assessed its effects on fMRI-measured responses. We analyzed responses to positively or negatively valenced faces when participants were required to amplify attention to one or the other class. We investigated differential effects of congruence by comparing positive (+ Context & + Stimuli) to negative (- Context & - Stimuli) conditions. We suggest that the observed effects reflect the salience of rewarding and punitive stimuli on brain responses in adolescence, therefore focusing the study on youth (9-21 yrs). **Methods:** fMRI, using a mixed-block design, was collected (3T, TR/TE: 2000/30 ms, 30 slices, Voxel size: 3.75 x 3.75 x 3.8 mm^3, FOV 64 x 64 mm^2). 35 youth performed the task. A block of trials began with instructions indicating the affective context (positive or negative). Next, participants were shown a series of faces (1 Hz, jittered ISI) with letters ("A" or "X") imposed

on the bridge of the nose. "X" was a target only if the valence of the face was consistent with the valence of context. SPM8 was used in event related analyses to model responses to congruent events. Each participant contributed two contrast maps (positive and negative) modeled at a second level using pair-sample t-tests. **Results:** Congruence amplified cortical responses, but relative to negative, the positive condition resulted in substantively increased modulation of cortical-limbic responses ($p<.05$). Increases were observed in a network of regions including the amygdala, basal ganglia, dorsal anterior cingulate, fusiform gyrus, hippocampus, orbitofrontal cortex, ventromedial prefrontal cortex, and thalamus. **Conclusions:** Amplified modulation in context and stimulus congruence suggests that coupling of top-down (attention-driven) and bottom-up (stimulus-driven) processing is important in driving brain responses. Moreover, positive congruence was evocative, pointing to the salience of positive stimuli and contexts (Todorov et al., 2008) on reward circuits in development. The effect of positive congruence may reflect sensitivity of the cortical-limbic system for social reward.

Disclosures: R. Manimalethu: None. **T. Bawa:** None. **K. Ramaseshan:** None. **M. Re:** None. **P. Brambilla:** None. **V. Diwadkar:** None.

Poster

731. Motivation and Emotion: Reward III

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Program#/Poster#: 731.19/CC52

Topic: F.03. Motivation and Emotion

Support: NWO VENI grant 016.135.023

AXA Research Fund fellowship 2011

Title: Central obesity is predicted by contrasting prefrontal and striatal BOLD responses to food words in a color-naming Stroop task

Authors: *L. K. JANSSEN[1], I. DUIF[1], I. VAN LOON[1], J. H. M. DE VRIES[2], R. COOLS[1,3], E. AARTS[1];
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Abstract: The motivating force of food can override homeostatic signals leading to loss of control and overconsumption. Elevated neural reward responses to food cues have been shown to

predict weight gain[1] and the likelihood of self-control failures in daily life, whereas prefrontal responses were found to be associated with successful resistance to temptations[2]. However, these findings were obtained using different types of tasks, probing reward and cognitive control functions respectively. In daily life, reward and cognitive control processes are likely to act simultaneously during the resistance of high caloric food. It is yet unclear whether processing of food stimuli in fronto-striatal regions within the same task can differentially predict obesity. Here, we used the food Stroop task to obtain neural measures of (overcoming) attentional bias to food stimuli. Previous studies have used the behavioral attentional bias effect, i.e. slower responding to the color of food words than to the color of matched neutral words, to predict obesity, but with mixed results[3]. Here, we tested 79 healthy participants with a wide body mass index range (BMI: 19-35 kg/m2) using fMRI. During scanning, participants performed a food Stroop task. In addition, we measured height, weight, and waist and hip circumference as measures of central obesity. Regression analyses showed that central obesity was best predicted by a combination of decreased dorsolateral prefrontal cortex and increased striatal BOLD responses to food versus neutral words during color-naming. These data suggest that attentional bias to food stimuli, perhaps leading to overconsumption and central obesity, is modulated by an interplay between striatal reward responses and top-down control from dorsolateral prefrontal cortex. References: [1] Stoeckel et al., NeuroImage, 2008; [2] Lopez et al., Psych Science, 2014; [3] Werhtmann et al., Proc of the Nutrition Society, 2014

Disclosures: **L.K. Janssen:** None. **I. Duif:** None. **I. van Loon:** None. **J.H.M. de Vries:** None. **R. Cools:** None. **E. Aarts:** None.

Poster

731. Motivation and Emotion: Reward III

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Program#/Poster#: 731.20/CC53

Topic: F.03. Motivation and Emotion

Support: NIDA Grant 027764

Title: Neural responses to cigarette and monetary gains and losses in deprived smokers

Authors: *A. H. LEWIS, H. MANGLANI, M. R. DELGADO;
Psychology, Rutgers Univ., Newark, NJ

Abstract: Research examining addicted populations posits that these individuals exhibit decreased sensitivity to non-drug rewards (e.g. Goldstein et al., 2007; Martin-Soelch et al., 2003;

Rose et al., 2012). For instance, deprived smokers show enhanced neural responses to the anticipation of cigarette rewards compared to monetary rewards (Sweitzer et al., 2014). One interesting question is how abstinence influences reward-related neural activity during both the receipt and loss of drug (cigarette) and non-drug (monetary) reinforcers. In the current study, we examined the effects of smoking deprivation on the anticipation and processing of both smoking and monetary-related gains and losses. Participants were deprived of smoking for 12 hours and engaged in a card-guessing paradigm (Delgado et al., 2000) that afforded the opportunity to earn both money and cigarette puffs that would be delivered at the end of the experiment. Notably, a willingness to pay (WTP) measure taken prior to the task was used to equate the cigarette and monetary outcomes in terms of value. For example, a participant who indicated a WTP of $0.20 for one cigarette puff played the card-game using those specific incentive values. Participants underwent four rounds of the task, two of which dealt cigarette outcomes and two of which dealt monetary outcomes. At the start of each trial, participants guessed whether the number on the back of a card was higher or lower than 5 (anticipation phase). Correct guesses led to monetary or cigarette gains while incorrect guesses led to monetary or cigarette losses (outcome phase). Preliminary neuroimaging analyses highlight the involvement of the insula during the anticipation phase, which showed greater activation when anticipating cigarette compared to monetary reinforcers. During the outcome phase, we focused on activity in the striatum, which typically shows a greater response to gains compared to losses in this task. Interestingly, this differential response was replicated for trials involving monetary outcomes, but was diminished for trials involving cigarette outcomes. Taken together, these findings highlight differential brain responses to both appetitive and aversive drug (cigarette) and non-drug (monetary) reinforcers in a deprived smoking population.

Disclosures: **A.H. Lewis:** None. **H. Manglani:** None. **M.R. Delgado:** None.

Poster

732. *In vivo* Imaging Methods

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 732.01/CC54

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Fast, non-motorized focus and drift correction for long-term spine imaging with an Electrical Tunable Lens

Authors: ***M. S. SMIRNOV**, A. FERRARI, R. YASUDA, L. YAN;
Max Planck Florida Inst. for Neurosci., Jupiter, FL

Abstract: During micron-scale photostimulation and imaging of neuronal activity, the presence of focal and lateral drift can often lead to errors in measurement and loss of data. We present a low-cost method to automatically correct for X, Y, and Z drift during live, two-photon imaging without relying on a motorized stage. With the use of an electrically tunable lens in the excitation path, we are able to rapidly and precisely alter focal distance, while changes in scan angle are used to shift the field of view in the XY plane. Correction in all three axes can be as large as 1000μm using a 20x objective. Quick and seamless switching between scan fields allows users to define multiple imaging/photostimulation regions, and automatic drift and focus correction insures that imaging can proceed unattended. All hardware is controlled through a user-friendly interface and accessible MATLAB code.

Disclosures: **M.S. Smirnov:** None. **A. Ferrari:** None. **R. Yasuda:** None. **L. Yan:** None.

Poster

732. *In vivo* Imaging Methods

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Program#/Poster#: 732.02/CC55

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: DARPA W911NF-14-2-0013

NIH PIONEER 8DPIHD075623

Title: Design of an implantable artificial dural window for chronic two-photon optical imaging in non-human primates

Authors: ***E. TRAUTMANN**[1], D. O'SHEA[1], S. SHRESTHA[2], S. LIN[3], S. RYU[4], K. SHENOY[3,5];
[1]Stanford Neurosciences, San Francisco, CA; [2]Stanford Mechanical Engin., San Francisco, CA; [3]Stanford Electrical Engin., San Francisco, CA; [4]Dept. of Neurosurg., Stanford Univ., San Francisco, CA; [5]Neurobio., Stanford Univ., Stanford, CA

Abstract: Optical functional imaging methods such as calcium imaging have become a powerful tool for investigating neural activity in-vivo. We present an implantable titanium chamber with silicone artificial dura, which enables two-photon (2P) calcium imaging in non-human primates (NHP). This chamber accommodates imaging with large, multiphoton objective lenses, and remains sealed, protecting the brain from the environment. In addition, we describe a stabilization system to restrict tissue motion while imaging during motor behaviors. Calcium

imaging presents several advantages over more traditional multi-electrode recordings, including the ability to genetically target specific cell types and to densely sample from every neuron within a recording volume. However, translating optical imaging techniques to awake, behaving macaques presents a set of unique challenges. First, the optical window must be designed around the large, high numerical aperture objective lenses typically required for 2P imaging. We developed a novel optical imaging chamber for NHP, which is compatible these lenses. Second, the imaging chamber must be durable enough to last for several months to years to align with trained NHP experimental timescales. Our design incorporates a replaceable window, which is sealed from the external environment to minimize immunoreactivity. Third, cardiac and respiratory rhythms induce cortical pulsations, making stabilization prerequisite for 2P imaging in NHP. In our case, a motor reaching task provides another source of potential brain motion, increasing the need for stabilization. To address this, we developed a stabilization system, which uses gentle pressure on the window to restrict total XY motion to ~5-10 μm for prolonged experiments. The work here describes a system that addresses each of these three key challenges, enabling the capture of stable, cellular resolution, 2P images of superficial motor cortex, and facilitating optical interrogation of neural activity using calcium reporters in future work. The applications of this artificial dural window are not limited to 2P calcium imaging. This implant design may benefit a number of experimental modalities, including single and multiphoton imaging of voltage and calcium reporters, optogenetics, or electrophysiology experiments using fragile silicon electrodes, high-density multielectrodes, or where precise localization relative to identifiable cortical landmarks is essential. We anticipate that this design may soon facilitate a new class of circuit and systems neuroscience experiments in behaving non-human primates.

Disclosures: **E. Trautmann:** None. **D. O'Shea:** None. **S. Shrestha:** None. **S. Lin:** None. **S. Ryu:** None. **K. Shenoy:** None.

Poster

732. *In vivo* Imaging Methods

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Program#/Poster#: 732.03/CC56

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: JSPS KAKENHI Grant No.26650107

Title: Improvement in focusing properties enables *in vivo* two-photon laser ablation in deep cortical regions of living mouse brain

Authors: *K. YAMAGUCHI[1,2], R. KITAMURA[1,2], R. KAWAKAMI[1,2], T. NEMOTO[1,2]; [1]Res. Inst. of Electronic Sci., Sapporo, Japan; [2]Grad. Sch. of Information Sci. and Technol., Sapporo, Japan

Abstract: Optical dissection of nerve fibers in the living mouse brain will provide insights into the functions of neural networks. Although dendrites of pyramidal neuron and axons spread across all cortical layers, the dissection using ultrashort pulse laser has achieved in superficial cortical region. This phenomenon is a result of degradation of laser focusing, especially in deep regions, due to refractive index mismatches between tissue, immersion liquid, and glass, as well as laser light scattering in the living brain. In this study, we achieved *in vivo* two-photon laser ablation at focal regions of the living brain under an optimized laser irradiation condition. For *in vivo* fluorescence imaging and laser ablation, adult Thy1-eYFP-H transgenic mouse were used. Surgeries were performed using the "open-skull method, in which a piece of skull bone is replaced with a round cover slip (φ4.2 mm), which serves as a cranial window. Under anesthesia, mice were placed in a two-photon microscopy system with a Tsunami near-infrared laser (Spectral Physics). First, under normal conditions, we confirmed that single nerve fibers were dissected in cerebral cortex layer II/III, and also observed dendritic degeneration *in vivo*. Next, in order to visualize single nerve fibers in deeper regions, we optimized the parameters of laser irradiation in these regions. To prevent degradation of laser power and focus in deeper layers, we examined the effects of beam diameter and refractive index of the immersion liquid. Thereby we found a suitable combination of a narrower laser beam diameter and an immersion liquid, and successfully achieved laser ablation of single dendrites of neurons in cerebral cortex over 500 μm from the brain surface. We hope that this technique will be useful for severing neural connections in cortical regions, with the goal of revealing brain functions.

Disclosures: K. Yamaguchi: None. **R. Kitamura:** None. **R. Kawakami:** None. **T. Nemoto:** None.

Poster

732. *In vivo* Imaging Methods

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant GM096884

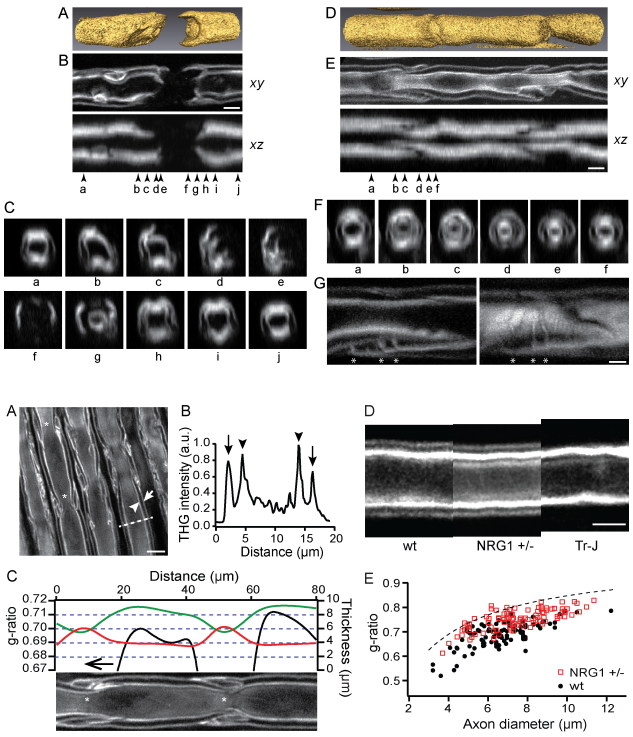
NIH Grant NS000001

NIH Grant NS26001

Title: Label-free imaging of Schwann cell myelination by third harmonic generation microscopy

Authors: *H. LIM[1], D. SHAROUKHOV[1], Y. ZHANG[3], J. L. SALZER[3], C. MELENDEZ-VASQUEZ[2];
[2]Biol., [1]Hunter Col., New York, NY; [3]Cell Biol. and Neurol., New York Univ. Sch. of Med., New York, NY

Abstract: Understanding the axon-glial cell interaction underlying myelination is hampered by the lack of suitable imaging techniques. Here we describe a new method, namely third harmonic generation microscopy (THGM), for label-free imaging of myelinating Schwann cells in live culture and *ex vivo* and *in vivo* tissue. Three-dimensional structure was acquired for a variety of compact and non-compact myelin domains, including juxtaparanodes, Schmidt-Lanterman incisures, and Cajal bands. Other subcellular features of Schwann cell that escape traditional optical microscopies were also visualized. We tested THGM for morphometry of compact myelin. Unlike current method based on electron microscopy, g-ratio could be determined along an extended length of myelinated fiber in the physiological condition. The precision of THGM-based g-ratio estimation was corroborated in mouse models of hypomyelination. Finally, we demonstrated the feasibility of THGM to monitor morphological changes of myelin during postnatal development and degeneration. The outstanding capabilities of THGM may be useful for elucidation of the mechanism of myelin formation and pathogenesis.



Disclosures: **H. Lim:** None. **D. Sharoukhov:** None. **Y. Zhang:** None. **J.L. Salzer:** None. **C. Melendez-Vasquez:** None.

Poster

732. *In vivo* Imaging Methods

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Program#/Poster#: 732.05/CC58

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: JSPS KAKENHI grant, No. 22113005

JSPS KAKENHI grant, No. 26242082

Title: *In vivo* two-photon imaging of hippocampal neurons in dentate gyrus using a newly developed a high-peak power 1064-nm light source based on a gain-switched laser diode

Authors: *K. SAWADA[1,2], R. KAWAKAMI[1,2,3], Y. KUSAMA[4], Y.-C. FANG[4], S. KANAZAWA[5], Y. KOZAWA[3,5], S. SATO[3,5], H. YOKOYAMA[3,4], T. NEMOTO[1,2,3]; [1]Grad. Sch. of Information Sci. and Technol., Sapporo / Hokkaido, Japan; [2]Res. Inst. for Electronic Science, Hokkaido Univ., Sapporo / Hokkaido, Japan; [3]Core Res. for Evolutional Sci. and Technol. (CREST), Japan Sci. and Technol. Agency (JST), Chiyoda-ku / Tokyo, Japan; [4]New Industry Creation Hatchery Ctr. (NICHe), Tohoku Univ., Sendai / Miyagi, Japan; [5]Inst. of Multidisciplinary Res. for Advanced Materials, Tohoku Univ., Sendai / Miyagi, Japan

Abstract: Conventional *in vivo* two-photon microscopy has revealed vital information about neural activity in relation to brain function, despite its limitations in imaging events at depths greater than several hundred micrometers from the surface of the brain. In a previous study, we clearly demonstrated that the feasibility of using a near-infrared picosecond high-power pulse laser for *in vivo* two-photon microscopy (Kawakami, et al., Sci. Rep. 2013). This laser enables us to visualize cortex pyramidal neurons spreading to all cortical layers, as well as hippocampal CA1 neurons, especially in young adult mice. In this study, we developed a novel two-photon microscope consisting of a 1064-nm gain-switched laser diode-based light source with average power above 4 W, pulse width of 7.5 picoseconds, repetition rate of 10 MHz, and a high-sensitivity photomultiplier tube for efficient detection of fluorescence. By applying this newly developed two-photon microscope to *in vivo* imaging, we were able to successfully visualize hippocampal neurons in dentate gyrus and panoramic views of CA1 pyramidal neurons and cerebral cortex, in both young adult and adult mice. Fine structures of dendrites in CA1 neurons could be visualized with a high peak-signal-to background ratio that could not be achieved by

titanium sapphire laser excitation. Furthermore, our system achieved multicolor imaging with neurons and blood vessels in the hippocampal region *in vivo* . Notably, we did not observe any photobleaching or photodamage within the area through which the laser light passed, including the cortex above the hippocampal area, even though the applied laser power used for imaging hippocampal neurons was about 500 mW. We hope that our two-photon microscopy system will be applicable to investigations of various neural functions, including the morphological changes undergone by neurons during physiological phenomena.

Disclosures: **K. Sawada:** None. **R. Kawakami:** None. **Y. Kusama:** None. **Y. Fang:** None. **S. Kanazawa:** None. **Y. Kozawa:** None. **S. Sato:** None. **H. Yokoyama:** None. **T. Nemoto:** None.

Poster

732. *In vivo* Imaging Methods

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH grant 2R01MH083686

Title: Brain temperature changes under near-IR laser illumination

Authors: ***A. SONG**, S. Y. THIBERGE, N. T. EMERSON, H. YANG, D. W. TANK; Princeton Univ., Princeton, NJ

Abstract: Two-photon microscopy and two-photon optogenetic stimulation are widely used techniques for imaging and manipulating activity of neurons *in vivo*. Recent developments in these areas include improved methods for volumetric imaging, strategies for imaging deeper brain structures, and techniques for cellular resolution photostimulation. These advances, however, generally increase the average power used for two-photon excitation to hundreds of mW, which may cause significant tissue heating. Heating can cause changes in neuronal function, and at high levels, it can cause tissue damage. For this reason, we explored the heating effects due to one-photon absorption of near-IR laser illumination used for two-photon excitation. We examined near-IR laser heating effects in the brains of awake mice using an optical cranial window with a small silicone elastomer plug, allowing access for injections and probes. A hypodermic probe thermocouple measured the amount and distribution of heating in the mouse brain at different positions and depths outside the scanned area. We explored a variety of different parameters, including wavelength (800nm-1060nm), power, two-photon excitation strength, and imaged area (200μm-2mm). We find that laser heating can be significant, causing

temperature increases of several degrees Celsius with high average power (hundreds of mW). This effect is primarily due to one-photon absorption, and spreads out from the imaged area with a 30-60s timescale. A previous study has demonstrated that cranial windows typically used for imaging experiments can decrease the brain surface temperature by up to 10°C [1]. The near-IR illumination temperature increases we measured were produced relative to this altered baseline temperature. We modeled the temperature distribution across the brain using a 3D thermal diffusion model with terms for laser heating (source), blood flow (sink), and convective cooling from the cranial window (sink). Finally, we have begun to explore the use of ratiometric temperature-sensing quantum dots to record local temperature fluctuations within the scanned region. In general, our experiments and model suggest limits on average power used during two-photon based techniques, and highlight considerations that must be taken to ensure neuronal homeostasis. [1] Kalmbach AS, Waters J. Brain surface temperature under a craniotomy. Journal of Neurophysiology 108: 3138-3146, 2012.

Disclosures: **A. Song:** None. **S.Y. Thiberge:** None. **N.T. Emerson:** None. **H. Yang:** None. **D.W. Tank:** None.

Poster

732. *In vivo* Imaging Methods

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Army Research Office

Corning Research

NIH Grant 5SC1HD068129

NIH Grant 2G12 RR003060-26A1

Title: Brain transmission in four optical windows of near infrared spectral regions from 700 to 2500 nm for deep optical imaging

Authors: L. SHI[1], L. SORDILLO[1], *A. RODRIGUEZ-CONTRERAS[2], R. ALFANO[1]; [1]Physics, Inst. for Ultrafast Spectroscopy and Lasers, CCNY, New York, NY; [2]Biol., CCNY, CUNY, New York, NY

Abstract: Near-infrared (NIR) radiation has been used for deep imaging inside brain by 1-Photon and 2-Photon imaging at the wavelength ranging from 650 - 950 nm, which is also called the “therapeutic window”. It is well known that scattering blurs and absorption reduces the photons for imaging. Using longer wavelengths will reduce scattering. However, longer wavelengths in far NIR region have been overlooked due to a lack of suitable NIR-CMOS imaging detectors. The focus of this research is to introduce 3 new optical windows beyond the therapeutic window, and to demonstrate their potential for deep imaging. Optical attenuation measurements were obtained by using the Cary 500 scan UV/VIS/NIR spectrophotometer in the spectral range from 400 to 2,500 nm. The transmission lengths (Lt) were measured in rat brain tissues (thicknesses 50 - 200 µm) in the second (1,100 - 1,350 nm), the third (centered at 1700 nm), and the fourth (centered at 2,200 nm) optical tissue windows, respectively. The transmission vs. thickness of tissue was measured and compared theoretically (results not shown). The total attenuation length (Lt) in brain tissues is shown in Figure 1. The Lt is the longest in the third optical tissue window, indicating that the wavelength around 1700 nm potentially provides the largest penetration depth. We conclude that the third optical tissue window (centered at 1700 nm) is ideal for deep imaging with relatively less scattering or absorption for multiphoton excitation and confocal one photon imaging applications.

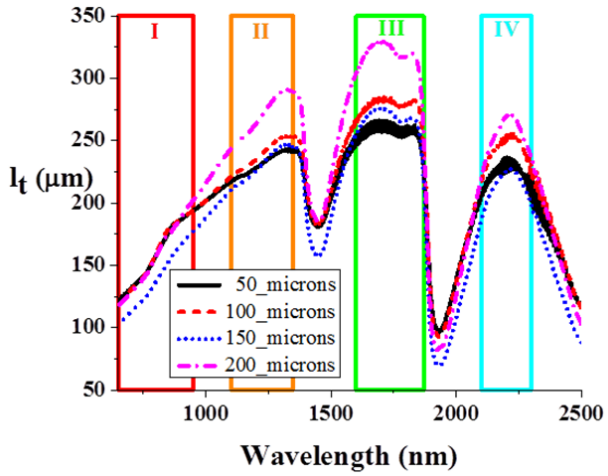

Fig. 1. Spectra of the total attenuation lengths (Lt, µm) from rat brain tissue thickness of 50 µm, 100 µm, 150 µm and 200 µm using the optical tissue window I, II, III and IV.

Disclosures: **L. Shi:** None. **L. Sordillo:** None. **A. Rodriguez-Contreras:** None. **R. Alfano:** None.

Poster

732. *In vivo* Imaging Methods

Location: Hall A

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Support: Grant-in-Aid for JSPS Fellows Number 15J10687

The Cooperative Study Program of National Institute for Physiological Sciences

The VLSI Design and Education Center (VDEC), University of Tokyo, in collaboration with Cadence Design Systems, Inc.

Title: Improvement of green fluorescence imaging system based on implantable CMOS imaging device for freely moving mice

Authors: *Y. SUNAGA[1], H. YAMAURA[2], M. HARUTA[1], T. YAMAGUCHI[1], M. MOTOYAMA[1], Y. OHTA[1], H. TAKEHARA[1], T. NODA[1], K. SASAGAWA[1], T. TOKUDA[1], Y. YOSHIMURA[2], J. OHTA[1];
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Abstract: To observe neural activities in the brain of an animal, fluorescence imaging technologees are widely used. Especially, autofluorescence of mitochondrial flavoprotein is attarctive because its intensity is changed by neural activities without dying neural cells nor introduction of genetically modification [1]. In this study, we report details of an implantable imaging device for freely moving mice, and also report experimental methods and results of *in vivo* fluorescence imaging experiments. To observe fluorescence in a mouse brain under freely moving condition, we have proposed a fluorescence imaging technique based on an implantable (CMOS) imaging device. By implanting a dedicated CMOS image sensor integrated with a fluorescence filter and LEDs as excitation light sources directly into a mouse brain, neural activity imaging based on fluorescence can be performed even under freely moving conditions. In our previous works, we have developed an implantable imaging device for green fluorescence imaging, and achieved to improve the detection sensitivity of green fluorescence [2]. Recently, we have succeeded in observing visually evoked flavoprotein fluorescence changes across 25 trials in the visual cortex of a tethered mouse using the implantable imaging device [3]. In this experiment, a full-field sinusoidal grating with vertical orientation was applied to activate visual cortex. To apply this technology to observing flavoprotein fluorescence in a freely-moving condition where noise may increase, we have developed a new implementation method to reject excitation light more efficiently. The previous implementation method has two issues. One is to reduce leakage of excitation light. The imaging area near LEDs was especially influenced by leakage of excitation light. In this work, we succeeded in reducing leakage of excitation light by filling space between LEDs and a sensor chip with the same component of a fluorescence filter.

The other is that it is difficult to fabricate a flat fluorescence filter. The fluorescence filter was directly formed on the small sensor chips by a spin-coating method, which easily produces some bumps in the corners of the filter. In this work, we succeeded in fabricating flatter filter as follows; the filter is separately made on a cover grass which has lager area than the chip, and then is cut into a chip-size with a laser. Finally, the fabricated filter is put on the sensor surface. We are now working to confirm the performance of the improved device. [1] K. Shibuki et al. J. Physiol.. 549(3), 919927, 2003. [2] Y. Sunaga et al., IEEE BioCAS 2014, Lausanne, Swiss. [3] Y. Sunaga et al., to be presented in M&BE 2015, Tokyo, Japan.

Disclosures: **Y. Sunaga:** None. **H. Yamaura:** A. Employment/Salary (full or part-time):; National Institute for Physiological Sciences. **M. Haruta:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **T. Yamaguchi:** None. **M. Motoyama:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **Y. Ohta:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **H. Takehara:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **T. Noda:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **K. Sasagawa:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **T. Tokuda:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **Y. Yoshimura:** A. Employment/Salary (full or part-time):; National Institute for Physiological Sciences. **J. Ohta:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology.

Poster

732. *In vivo* Imaging Methods

Location: Hall A

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Program#/Poster#: 732.09/CC62

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: LASERLAB-EUROPE 284464

Human Brain Project 604102

POR-CreO 2007-2013 action (SMAG project)

Flagship Project NANOMAX

Title: Label-free NIR reflectance imaging as a complimentary tool for two-photon fluorescence brain imaging

Authors: *A. ALLEGRA MASCARO[1,2], I. COSTANTINI[1], E. MARGONI[1], G. IANNELLO[3], L. SACCONI[1,2], F. S. PAVONE[1,4,5];
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Abstract: *In vivo* two-photon imaging combined with targeted fluorescent indicators is nowadays extensively used for attaining critical insights into brain functionality and structural plasticity. Additional information might be gained from back-scattered photons from the NIR laser without introducing any exogenous labelling. Here, we describe a complimentary and versatile approach that, by collecting the reflected NIR light, provides structural details on the myelinated axons and blood vessels in the brain, both in fixed samples and in live animals under a cranial windows. Indeed, by combining NIR reflectance and two-photon imaging of a slice of hippocampus from a Thy1-GFPm mouse, we show the presence of randomly-oriented axons intermingled with sparsely fluorescent neuronal processes. The back-scattered photons guide the contextualization of the fluorescence structure within brain atlas thanks to the recognition of characteristic hippocampal structures. Interestingly, myelin formations allowed the label-free detection of axonal elongations over the layer 2/3 of mouse cortex under a cranial window *in vivo*. Finally, blood flow can be measured in live preparations, thus validating label free NIR reflectance as a tool for monitoring haemodynamic fluctuations. The prospective versatility of this label-free technique complimentary to two-photon fluorescence microscopy is demonstrated in a mouse model of photothrombotic stroke in which the axonal degeneration and blood flow remodelling has been investigated.

Disclosures: A. Allegra Mascaro: None. **I. Costantini:** None. **E. Margoni:** None. **G. Iannello:** None. **L. Sacconi:** None. **F.S. Pavone:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSFC Grant 91232710

Title: Novel skull optical clearing facilitates imaging *in vivo* of Neural Circuitry in infantile mice cerebral cortex

Authors: Y. ZHAO, C. ZHANG, *T. XU, D. ZHU;
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Abstract: Two-photon microscopic *in vivo* imaging of cerebral cortex in fluorescent protein transgenic mice have shown great potential for understanding of a broad array of neurobiological phenomena, especially the dynamics of individual synapses and the functional organization of cortical maps in learning and memory and various brain diseases. Regarding the imaging performance always suffers from the turbid skull above the cortex, various cranial windows, such as remove skull, cranial window, thinned-skull cranial window, and polished and reinforced thinned skull window were developed to improve imaging. However, these models are not options available for infantile mice due to the vulnerability of young animals. Actually, the critical period for cortical development for young mice (2-6 weeks) is always a matter of concern. Specifically, the peak of anatomical and physiological plasticity of dendritic spines occurs during the third week. Up till now, the plasticity of neural infactile mice circuitry less than one month has not been studied *in vivo* very well. In this work, we developed an innovative skull optical clearing method, which can make the skull transparent by topically treatment with skull optical clearing agents instead of craniotomy. Through the clearing skull, we could image the dendritic protrusions of infactile mice *in vivo*. Our preliminary data showed the high dynamics of cortex circuitry in three-week old mice. In addition, the safety of skull optical clearing method was approved by dynamical monitoring the blood flow and observation of inflammation reaction.

Disclosures: Y. Zhao: None. **C. Zhang:** None. **T. Xu:** None. **D. Zhu:** None.

Poster

732. *In vivo* Imaging Methods

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Topic: G.03. Staining, Tracing, and Imaging Techniques

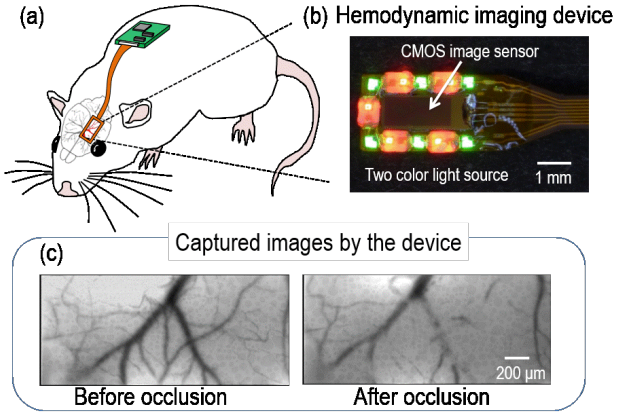
Support: JSPS KAKENHI 26882027

The VLSI Design and Education Center (VDEC), University of Tokyo, in collaboration with Cadence Design systems, Inc.

Title: An implantable hemodynamic imaging device for revealing relation between a blood flow and brain activity in animal behavior

Authors: *M. HARUTA, Y. SUNAGA, T. YAMAGUCHI, H. TAKEHARA, Y. OHTA, M. MOTOYAMA, H. TAKEHARA, T. NODA, K. SASAGAWA, T. TOKUDA, J. OHTA;
Nara Inst. of Sci. and Technol., Ikoma / Nara, Japan

Abstract: We have developed an implantable hemodynamic imaging device for detecting a blood flow and brain activity in a small animal brain in a same time (Fig. (a)). The device includes a CMOS image sensor and LEDs for illuminations on a flexible substrate. A photo of the device is shown in Fig. (b). We can simultaneously measure a blood flow and brain activity by using two-color, or green and red LEDs on the device. A green LED emits at the peak wavelength of 535 nm where one of the peaks of hemoglobin absorption in the blood exist. We can measure a blood flow in a blood vessel with the LED, and observe both a blood flow velocity and a direction in a brain. A red LED emits at the peak wavelength of 605 nm where absorbance is different between oxy-hemoglobin and hemoglobin in the blood. We can measure brain activity with the red LED. Our device has advantage that it can measure hemodynamic signals under the implant state in a small animal, and such advantage provides a cerebrovascular disease study with an effective method. For measurement of the cerebrovascular disease study, magnetic resonance imaging (MRI) and computed tomography (CT) were generally used. However, these methods were not useful for a behavior study of a small animal such as a mouse. Our device can measure the blood flow and the brain activity in the behavior experiment of a small animal. This makes it possible to reveal relations between the blood flow, the brain function and the animal behavior. In this study, we successfully measured blood flow changes before and after a photothrombotic brain ischemia. Figure (c) shows images captured by the device before and after the ischemia. In the next step, we are planning to measure the blood flow and the brain activity in animal behavior to reveal relation between the cerebrovascular disease and the brain function.



Disclosures: **M. Haruta:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **Y. Sunaga:** None. **T. Yamaguchi:** None. **H. Takehara:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **Y. Ohta:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **M.**

Motoyama: A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **H. Takehara:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **T. Noda:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **K. Sasagawa:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **T. Tokuda:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology. **J. Ohta:** A. Employment/Salary (full or part-time):; Nara Institute of Science and Technology.

Poster

732. *In vivo* Imaging Methods

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 732.12/CC65

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: JSPS KAKENHI Grant No.26650107

Title: Quantitative evaluation of the resolution of *in vivo* two-photon microscopy by imaging of single fluorescent beads in living mouse brain

Authors: *R. KITAMURA[1,2], K. YAMAGUCHI[1,2], R. KAWAKAMI[1,2], T. NEMOTO[1,2]; [1]Res. Inst. for Electronic Sci., Sapporo / Hokkaido, Japan; [2]Grad. Sch. of Information Sci. and Technology, Hokkaido Univ., Sapporo / Hokkaido, Japan

Abstract: Synaptic plasticity induces morphological changes in post-synaptic structures, including dendritic spines, which have been implicated in information processing by neural network. Visualization of morphological changes in the living mouse brain will provide insight into brain functions such as learning or memory. Usually, observation of dendritic spines in living specimens is performed by *in vivo* two-photon microscopy, because of its high spatial resolution and deep imaging capability. In our previous studies, we found that penetration depth could be improved by changing the diameter of the irradiation excitation laser beam. However, the resolution under these conditions was not determined, because the focal spot size of the excitation light was not measured precisely. In general, the resolution of a laser scanning microscope is correlated with the focal spot size. This size is sometimes evaluated by measuring full width at half maximum (FWHM) of a structure with a known shape (e.g., a fluorescent bead) that is smaller than the diffraction limit. In this study, we injected fluorescent beads into the living mouse brain, and succeeded in *in vivo* two-photon imaging of single beads at various depths in the brain. Next, we estimated the resolutions by measuring FWHM from single-bead images, and then examined how the resolutions depended on the laser diameter and refractive

index of the immersion liquid.The results showed that FWHM on narrower laser beam diameter with higher average power was larger than that on full-filled condition. However, degradation of resolution was not remarkable for imaging of cortical neurons. Furthermore, differences between the refractive indices of the immersion liquids and the object being imaged resulted in degradation of focus. By decreasing mismatches in the refractive index , we achieved higher resolution at deeper regions of living mouse cerebral cortex. Thus, adjustment of the observation conditions to match the optical properties of the brain improves resolution at high penetration depth without requiring the use of a special device. We hope that this technique will be applicable to investigations of various neural functions, including the morphological changes undergone by neurons during physiological phenomena.

Disclosures: **R. Kitamura:** None. **K. Yamaguchi:** None. **R. Kawakami:** None. **T. Nemoto:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Welcome Trust

ERC

Title: High speed 3D two-photon imaging and real time movement correction *in vivo* with a second generation Acousto-Optic Lens Microscope

Authors: *V. GRIFFITHS[1], S. K. M. NADELLA[2], H. ROS[2], G. KONSTANTINOU[2], T. KOIMTZIS[2], C. BARAGLI[2], P. A. KIRKBY[2], A. R. SILVER[2];
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Abstract: Neuronal signals are brief and sparsely distributed in 3D across synapses, neurons and networks. Mechanical focussing used in conventional two-photon microscopes therefore limits functional measurements of brain activity. To overcome this limitation several groups have developed two-photon microscopes based on Acousto Optic Lens (AOL) technology, which allow high speed 3D random access point measurements. However, the performance of current AOL microscope design is not ideal for *in vivo* applications, because fast full frame raster scanning is limited to the natural focal plane and pointing to voxels sequentially is inefficient for

creating the 3D z-stacks required. Moreover, rapid 3D random access point measurements are prone to movement artefacts, which unlike raster-scan imaging, cannot be corrected post hoc. Previous studies have shown lateral movement artefacts of up to 10μm in awake, head restrained mice. This makes random access point measurements from dendrites and spines challenging in awake animals, because brain motion must be detected and the motion-corrupted recordings discarded. To address these serious limitations we have developed a second generation 3D AOL microscope with a fast Field Programmable Gate Array (FPGA) control and acquisition systems. This allows the AOL to perform raster scanning in any focal plane within the field of view (FOV) and voxel dwell times down to 50ns. Full resolution (512x512 voxel) imaging can be performed at 39Hz, while reduced resolution (128x128) can be performed at 252Hz. The ability to jump to any location in the 3D FOV within 24.5 μs enables the AOL to scan planes that are tilted in Z, allowing features such as dendrites that span several Z planes, to be monitored with a single tilted plane at full imaging speed. Alternatively, multiple planes of interest, sparse 'patches' or multiple small volumes distributed in the 3D FOV can be imaged. By linking up real time movement tracking, implemented in the acquisition system, with the control FPGA, we have implemented real time movement detection and correction. The system performance was quantified using fluorescent beads mounted on a piezoelectric stage. Continuous sinusoidal movement of +/-10μm could be compensated with an error of <1μm for frequencies up to 20Hz. For movements of up to +/-8μm and frequencies of up to 20Hz the AOL microscope could consistently monitor a 1μm beads with random access point measurements. Preliminary experiments on awake, head restrained mice, using the somata of cerebellar interneurons expressing GFP as reference objects, suggest that movement can be compensated in real time to within 0.5μm, even during periods of intense running.

Disclosures: **V. Griffiths:** None. **S.K.M. Nadella:** None. **H. Ros:** None. **G. Konstantinou:** None. **T. Koimtzis:** None. **C. Baragli:** None. **P.A. Kirkby:** None. **A.R. Silver:** None.

Poster

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Support: NSF IGERT

Kavli Foundation

MURI W911NF-12-1-0594

Wallace H. Coulter Foundation

NSF 0954796

R01NS063226

R01 NS076628

Title: SCAPE microscopy of the awake, behaving mouse brain

Authors: *E. M. HILLMAN[1], V. VOLETI[1], C. O. LACEFIELD[2], M. B. BOUCHARD[1], W. LI[1], R. BRUNO[2];
[1]Biomed. Engineer, [2]Neurosci., Columbia Univ., New York, NY

Abstract: Swept, confocally-aligned planar excitation (SCAPE) microscopy is a new approach to fast 3D microscopy of the living brain which combines light-sheet optical sectioning with confocal descanning. SCAPE uses a single, stationary objective lens and can image at rates exceeding 20 volumes per second. This unique imaging geometry makes SCAPE suitable for high-speed 3D imaging of cellular function in the awake, behaving mouse brain. In recent experiments we have demonstrated that, despite its current implementation with 488 nm single-photon excitation, SCAPE can image to depths of over 250 microns in the intact, in-vivo mouse cortex at up to 40 volumes per second. Results show dynamics of both local and global dendritic spikes across identifiable apical tufts of layer 5 neurons expressing GCaMP6F. Animals are simultaneously monitored to track stimulus delivery, behavioral responses to stimulation and spontaneous behavior. SCAPE is also well suited to imaging small organisms such as *Drosophila* melanogaster and zebrafish, and can capture cellular function and structure during free movement. Improving SCAPE for in-vivo mouse brain imaging will include extending this penetration depth and resolution through the use of longer excitation wavelengths, red-shifted proteins, structured light approaches and two-photon implementations. Approaches to analysis and visualization of this high-speed 4-dimensional data are also under development. Our latest developments and results will be presented.

Disclosures: **E.M. Hillman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent issued on technology. **V. Voleti:** None. **C.O. Lacefield:** None. **M.B. Bouchard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor on issued patent. **W. Li:** None. **R. Bruno:** None.

Poster

732. *In vivo* Imaging Methods

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant DC03180

Title: Flexible, nimble, and quiet two-photon microscope platform for auditory functional imaging of awake marmosets

Authors: *X. SONG[1], Y. GUO[2], X. LI[2], X. WANG[2];
[1]Johns Hopkins University. Dept. of Biomed. Engin., Baltimore, MD; [2]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Two-photon laser scanning microscopy (TPLSM) has been widely employed to study neuronal population functions *in vivo*. The standard implementation of TPLSM utilizes a pair of galvanometer mirrors to scan the imaging beam. However, this mechanical oscillation introduces a remarkable acoustic noise bearing a distinct pitch. This unwanted noise as well as the cooling noises from a Ti:Sapphire laser system, introduces potential auditory artifacts which haven't been carefully investigated in previous studies. Alternatively, a beam scanner based on acousto-optical deflector (AOD) enables fast, silent and random-access scanning; yet a pure random-access scanning mode might be vulnerable to movement artifacts and thus limits its imaging use in awake animals. We have designed and built a system capable of flexible and nimble two-photon AOD scanning imaging in awake animals (FANTASIA). We isolated the laser cooling system outside a double-walled acoustic testing chamber. Such a system can perform (1) 2D random-access scanning at a speed of 50,000 points per second; or (2) 2D raster scanning at video rate, or (3) even 3D multi-layer scanning without moving the objective. To test the system, we injected adeno-associated virus carrying GCaMP and GFP into the cortex of a marmoset monkey (callithrix jacchus), a highly vocal non-human primate species, which was implanted with an artificial dura based optical window and imaged under awake condition. Clear cellular structures could be resolved. Both scanning flexibility and nimbleness were achieved without generating noticeable acoustic noise. This research is supported by an NIH grant (DC-03180).

Disclosures: **X. Song:** None. **Y. Guo:** None. **X. Li:** None. **X. Wang:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant 1R01MH107238-01

Title: *In vivo* imaging of zebrafish brain structure and function with light sheet microscopy

Authors: *T. V. TRUONG[1], A. ANDREEV[1], D. B. HOLLAND[1], J. M. CHOI[1], S. MADAAN[1], W. DEMPSEY[2], G. GROSS[2], D. B. ARNOLD[2], K. CZAJKOWSKI[3], C. KESSELMAN[3], S. E. FRASER[1];
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Abstract: Light sheet microscopy, also known as Selective Plane Illumination Microscopy (SPIM), has emerged in recent years as the modality of choice to deliver high, balanced performance in resolution, speed, and penetration depth, while minimizing photo-induced damages (fluorophore bleaching and cellular toxicity). We have used SPIM to image both the structure and function of live, intact, larval zebrafish brains. In one-photon (1p) excitation mode, SPIM delivers the high signal rate and signal-to-noise ratio necessary for us to visualize synapses that have been fluorescently labeled, at endogenous concentration, with recombinant probes. In two-photon (2p) excitation mode, SPIM delivers higher penetration depth than the 1p-counterpart, though with lower signal rate (due to lower 2p absorption cross section), but still is more than 10 times faster than conventional 2p point-scanning microscopy. The near-infrared light of 2p excitation, invisible to most animals' eyes, is critical to allow observation of brain activity without perturbing the animal's behavior. Furthermore, the minimal photo-induced damage allows us to continuously record for extended periods of twelve hours or more. Together, the ability to image both the structure, at synaptic resolution, and function, at whole brain coverage, lays the foundation for our ongoing studies to understand the information coding and processing involved in learning and memory.

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Poster

732. *In vivo* Imaging Methods

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 732.17/CC70

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Fast three-dimensional imaging of neuronal assemblies in the mouse visual cortex using genetically-encoded neuronal indicators and two-photon microscopy

Authors: *G. SZALAY[1], J. LINDA[1], G. KATONA[1], P. MAÁK[2], M. VERESS[2], B. RÓZSA[1,3]; [1]MTA KOKI, Budapest, Hungary; [2]Dept. of Atomic Physics, Budapest Univ. of Technol. and Econ., Budapest, Hungary; [3]Pázmány Péter Catholic Univ., The Faculty of Information Technology, Hungary

Abstract: The understanding of brain computation requires novel methods that read out neural activity on different spatial and temporal scales. Two-photon calcium imaging is a powerful means for monitoring the activity of distinct neurons in brain tissue *in vivo*. Genetically-encoded fluorescent calcium sensors are widely used to image neural activity; therefore we imaged calcium indicator-expressing neurons in the mouse visual cortex (V1) through a cranial window *in vivo*. During *in vivo* imaging movement artifacts caused by heartbeat and breathing artifact poses other challenges. In this work we present multiple 3D, acousto-optical, two-photon laser-scanning technologies to monitor neuronal activity at different scales with genetically-encoded calcium indicators in a near-cubic-millimeter scan range (up to 700 × 700 × 1,400 μm^3), with a high scanning speed (up to 1 Mhz), with high (<500 nm) resolution in the center core, and less than 1.9 x 1.9 x 7.9 μm^3 resolution throughout the whole scanning volume. We used volumetric random-access calcium imaging of spontaneous and visual stimulation-evoked activity from hundreds of neurons. We applied 3D trajectory scanning technique to measure multiple dendritic segments *in vivo* with motion compensation possibility. Finally we introduced an expansion of trajectory scanning technique to monitor the activity from more than 100 cells *in vivo* while obtaining information for motion correction.

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Poster

732. *In vivo* Imaging Methods

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Program#/Poster#: 732.18/CC71

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Multilayer cortical imaging in freely behaving animals

Authors: S. GULATI, V. CAO, P. JOSHI, *S. L. OTTE;
Inscopix, Palo Alto, CA

Abstract: The ability to study cortical function in behaviorally-relevant contexts is essential to dissecting neural network dynamics underlying cognition, attention, and sensory-motor integration. The evolutionary recent six-layered neocortex is the seat for many of these higher-order brain functions. It is thus of paramount importance to understand how the intra and inter-laminar flow of information in this anatomically complex region of the active brain regulates these functions. This requires the technological capability to simultaneously image multiple layers of the necortex at cellular resolution (and with cell-type specificity) during active behavior. Until now, such imaging was limited to either *in vitro* cortical slices, which lack physiological connections, or to *in vivo* two-photon preparations, which requires animal immobilization, precluding analysis during active, naturalistic behavior. Here, we pair Inscopix's nVista head-mounted, miniaturized microscope with 1mm chronically implanted glass microprisms to simultaneously record the calcium activity of upto ~500 neurons across all six layers of the somatosensory cortex during naturalistic behavior. The calcium dynamics of individual cells were monitored over time and across behavioral states. This study allowed us to compare the intra and inter-layer responses of individual cell responses during a novel object recognition task. These experiments extend the reach of *in vivo* calcium imaging to chronic, simultaneous monitoring of entire cortical columns during free behavior.

Disclosures: **S. Gulati:** A. Employment/Salary (full or part-time):; Inscopix. **V. Cao:** A. Employment/Salary (full or part-time):; Inscopix. **P. Joshi:** A. Employment/Salary (full or part-time):; Inscopix. **S.L. Otte:** A. Employment/Salary (full or part-time):; Inscopix.

Poster

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ERC Advanced Grant to A.K.

Title: *In vivo* deep two-photon brain imaging with a red-shifted fluorometric Ca^{2+} indicator

Authors: *C. H. TISCHBIREK, A. BIRKNER, H. JIA, B. SAKMANN, A. KONNERTH; Inst. of Neuroscience, TU Munich, Munich, Germany

Abstract: *In vivo* Ca^{2+} imaging of neuronal populations in deep cortical layers has remained a major challenge, as the recording depth of two-photon microscopy is limited due to scattering and absorption of photons in brain tissue. A possible strategy to increase the imaging depth is the use of red-shifted fluorescent dyes, as scattering of photons is reduced at long wavelengths. Here, we tested the red-shifted fluorescent Ca^{2+} indicator Cal-590 for deep tissue and dual-color two-photon imaging experiments *in vivo*. Cal-590 has a maximum for two-photon excitation at a wavelength around 1050 nm and a maximum emission wavelength at 590 nm. To explore the potential of Cal-590 for measurements of neuronal activity in deep cortical layers of the mouse brain, we used bulk loading of the acetoxymethyl (AM) ester version of Cal-590 to label populations of neurons in layers 5 and 6, respectively. Combined two-photon imaging and cell-attached recordings revealed that, despite the relatively low affinity of Cal-590 for Ca^{2+} (Kd = 561 nM), single action potential-evoked Ca^{2+} transients were discernable with a good signal-to-noise ratio in most bulk loaded neurons. We were able to record spontaneous Ca^{2+} transients with rapid kinetics in the six layers of the cortex at depths of up to -900 μm below the pial surface. Similar results were recorded for individual neurons electroporated with Cal-590, with dendritic rise and decay times sufficient to distinguish the peaks of individual Ca^{2+} transients even for high-frequency trains (100 Hz) of action potentials. In addition to the deep imaging experiments, we used Cal-590 for multi-color functional imaging experiments in combination with other Ca^{2+} indicators. Ca^{2+} transients in the dendrites of an individual OGB-1-labeled neuron and the surrounding population of Cal-590-labeled cells were recorded simultaneously on two spectrally separated detection channels. We conclude that the red-shifted Ca^{2+} indicator Cal-590 allows acute *in vivo* two-photon Ca^{2+} imaging experiments in all layers of the mouse cortex as well as multi-color functional imaging experiments.

Disclosures: C.H. Tischbirek: None. **A. Birkner:** None. **H. Jia:** None. **B. Sakmann:** None. **A. Konnerth:** None.

Poster

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Corning research

Title: Transmission of Laguerre Gaussian, Bessel, and Gaussian beams through rat brain with different orbital angular momentum

Authors: *L. SHI[1,2], W. WANG[2], R. GOZALI[2], M. LAVERY[3], P. MARQUES[2], A. RODRÍGUEZ-CONTRERAS[1], R. ALFANO[2];
[1]Biol., City Col. of New York, New York, NY; [2]Inst. for Ultrafast Spectroscopy and Lasers, New York, NY; [3]Sch. of Physics and Astronomy, Univ. of Glasgow, Glasgow, United Kingdom

Abstract: One of the key problems of optical imaging in neuroscience is to image deep within the brain. In addition, imaging resolution is always blurred due to scattering, which needs to be overcome by selecting the appropriate coherent ballistic component. One solution is to choose a wavelength in one of the four optical windows within the near-infrared (NIR) region. Another method is to use the optimal types of spatial light beams. A salient property of the light is spatial wave front, which can be planar or helical. Examples of helical wave front are the Laguerre Gaussian (LG) and the Bessel vortex laser beams. These beams carry orbital angular momentum (OAM) and can be used to probe biological tissues, such as the brain, for deep imaging. Most recently, the transmission of tight focused vortex beams have been theoretically studied. Sun et al. proposed that the electric fields of vortex beams have more penetration capacity through the turbid media than that of no-vortex beams, and the larger the OAM of the vortex beams, the higher the penetration capacity. Ou's group calculated the scattering of the LG beam at different OAMs. They showed that the magnitude of the scattering intensity decreased as OAM increased, suggesting the increase of transmission intensity with OAM. Bessel beam, due to its property of self-healing, is hypothesized to be able to image deeper into the tissue than other beam types. Therefore, the objective of this study was to examine transmission effects of LG and Bessel beams with different OAMs in the brain tissue. We measured light scattering of the LG and Bessel vortex beams with different momentums in rat brain tissues *ex vivo*. These results were compared with that measured from a Gaussian (G) beam, a special case of LG beam with a planar wave front with OAM=0, which is commonly applied in brain imaging. The optical transmissions of LG, Bessel, and G beams were measured at different ratios of thickness (Z)/scattering distance (Ls), 1 to 117, where Ls was ~60 μm in rat brain. The entire transmission intensity after the beam propagating through the brain tissue was measured. Our experiments indicate that LG, Bessel, and G beams showed no significant difference of donut beams transmission in brain tissue at different OAMs in both the ballistic and diffusive regions. The transition points from the ballistic to diffusive region for different scattering media were located at Z/Ls=18 for all three beams in brain tissue. This preliminary data suggests that different types of laser beams have similar limitations for deep imaging in the brain.

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Poster

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Title: Optimizing all-optical interrogation for closed-loop control of neural circuits

Authors: *L. E. RUSSELL, A. M. PACKER, M. HAUSSER;
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Abstract: Manipulating activity patterns as they are occurring, moment-to-moment, in a flexible manner will permit new classes of experiment, enabling us to dissect the encoding and decoding schemes of mammalian neural circuits (Grosenick et al 2015). We have developed a novel approach for interrogation of neural circuits in which real-time analysis of population calcium imaging data is combined with near-instantaneous manipulation of the circuit via targeted two-photon optogenetic stimulation. Simultaneous calcium imaging and optogenetic manipulation is enabled through the use of a genetically encoded calcium indicator (GCaMP6s) and a red-shifted opsin (C1V1). A spatial light modulator allows multiple, defined cells to be targeted for concurrent and spatiotemporally precise photoactivation. Real-time analysis of the calcium imaging data is made possible by intercepting the raw acquisition data stream from our microscope, in combination with fast algorithms for activity detection in identified cells. In proof of principle experiments in mouse visual cortex, we demonstrate dynamic network perturbations guided by the activity patterns of multiple identified cells. We designated multiple “trigger” cells in a single field of view with corresponding sets of “targets”. When activity in any of the trigger cells exceeded threshold the corresponding targets were selectively activated. We have photostimulated up to 50 neurons simultaneously and validated the high-power handling of new SLM devices, which switch three times faster than in our previous work (Packer et al 2015).

Work is in progress to optimize beamlet delivery to wider fields of view as well as generate advanced analytical routines for dealing with the resulting datasets. This approach will enable new classes of experiment, such as: correcting erroneous network trajectories recorded from animals performing a decision-making task; inducing plasticity in neural circuits by recruiting cells into functional, existing or artificial ensembles; and processing functional connectivity maps in real time to update predictions and guide subsequent interrogation.

Disclosures: **L.E. Russell:** None. **A.M. Packer:** None. **M. Hausser:** None.

Poster

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Title: Quantitative phase imaging (QPI) of optogenetic signals in neurons

Authors: *T. KIM[1,2], P. SENGUPTA[1,2], M. U. GILLETTE[1,2], G. POPESCU[1,2];
[1]Univ. of Illinois At Urbana-Champaign, Champaign, IL; [2]Beckman Inst. for Advanced Sci. and Technol., Urbana, IL

Abstract: Fast signals in neurons play a central role in intercellular communication. These signals are generated through the ion channels on the plasma membrane, when the membrane potential increases and surpasses the threshold. This subtle and rapid event occur in millisecond timescale, which makes it extremely difficult to detect them without an expensive electrophysiology system. These systems also require a highly precise control during its operation and are limited to single point measurements. More recently, electrophysiology systems based on MEMS devices have been developed for multipoint detection of these signals. However, it remains challenging to do a wide-field imaging of these cells in action because of the required imaging speed and sensitivity. Recent advances in quantitative phase imaging (QPI) allowed unlabeled live biological specimens to be imaged with sub-nanometer sensitivity and diffraction limited resolution. A recently developed technique called Spatial Light Interference Microscopy (SLIM) has been applied for discovering the emergent behavior in human neuronal

networks and the intercellular dynamics and has proven its potential to be used in neuroscience [Z. Wang et al., Opt. Exp., 19, 1016 (2011) & M. Mir et al., Sci. Rep., 4, 4414 (2014)]. This technique, which allows for a wide-field imaging of live neuronal activities, therefore, can be used for a measurement of rapid and subtle action potentials in optogenetically engineered cells. By implementing a projector and imaging a pattern onto the sample plane, SLIM system acquires a light stimulation system, which is capable of lighting up individual cells. The cellular signals generated as a response to the light stimulation then are measured with a sub-nanometer sensitivity as they propagate through the cells. This new method of measuring action potentials in neurons can help understanding the cellular signaling mechanism and dynamics at a higher resolution.

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Poster

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Title: Real-time analysis of calcium imaging data

Authors: *A. MITANI, T. KOMIYAMA;
Dept. of Neurosciences, UCSD, La Jolla, CA

Abstract: *In vivo* Ca2+ imaging has become a powerful method to record the activity of ensembles of single neurons. Compared to electrophysiological recordings, it is more reliable to

identify the same set of neurons over a long period of time. In addition, with the expanding set of genetic tools, distinct cell types can be identified relatively easily. These advantages make calcium imaging well suited for closed-loop experiments, in which behavioral feedback is given in real time based on neural activity to probe brain plasticity. However, closed-loop experiments with calcium imaging are so far limited to only a few studies (Clancy et al (2014), Hira et al (2014)), partly due to technical challenges of real time processing of high-volume imaging data. Here we discuss our effort to streamline real time processing of full-field, 30Hz two-photon calcium imaging data from mouse neocortex. We developed a fast motion correction algorithm and integrated it into ScanImage4 as a plugin function. This system processes the same images saved for post-hoc analysis, and therefore there is no loss of information. In addition to closed-loop experiments discussed above, real-time motion correction would facilitate additional applications. First, task parameters could be tuned based on the response properties of imaged neurons. This has been a widely used approach in single-unit recording studies in monkeys to enhance the responses of the recorded neurons by selecting their preferred stimuli. Second, motion corrected images can be used to estimate drift in imaging plane automatically. Imaging sessions can last for hours, and the focal plane of the microscope relative to *in vivo* samples can shift over time. In most studies this issue is ignored or manually corrected, thus the deviation has to become large enough to be visible before corrected. We will show that automatic Z correction with Piezo-controller achieves more frequent and finer Z-plane adjustment and hence increases the stability of the imaging. This study will show the enhanced performance of the online image processing system in Ca2+ imaging and its promising applications.

Disclosures: **A. Mitani:** None. **T. Komiyama:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: The role of conserved polar amino acids at the transmembrane loop regons of a genetically encoded voltage sensor

Authors: M. SEPEHRI RAD[1], B. J. BAKER[1], *M. ALLAHVERDIZADEH[2], L. B. COHEN[3]; [1]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Thechnonoly, Seoul, Korea, Republic of; [2]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [3]Dept. of Mol. and Cell. Physiology, Yale Univ. Sch. of Medicine, New Haven, CT., New Haven, CT

Abstract: Designing genetically encoded voltage indicators (GEVI) to respond to specific voltage ranges will simplify the interpretation of optical recordings of neuronal activity *in vivo*. To identify potential regions of the voltage-sensing domain that could shift the voltage sensitivity, we aligned 183 voltage-gated sodium channels (Nav) from different organisms. Conserved polar residues were identified at multiple transmembrane-loop junctions in the voltage sensing domain. Similar conservation of polar amino acids were found in the voltage sensing domain of the voltage-sensing phosphatase (VSP) gene family. These conserved residues were mutated to nonpolar or oppositely charged amino acids in a GEVI that utilizes the voltage sensing domain of the VSP from *Ciona intestinalis* fused to the fluorescent protein, super ecliptic pHlorin (A227D) to determine their potential role in sensing voltage across the plasma membrane. Preliminary results show that some of the polar residues shift the optical response to more positive potentials. Interestingly, one conservative change of aspartic acid to glutamic acid abolished the membrane trafficking of the GEVI suggesting that the length of the side chain is important at the plasma membrane/extracellular interface.

Disclosures: **M. Sepehri Rad:** None. **B. J. Baker:** None. **M. Allahverdizadeh:** None. **L. B. Cohen:** A. Employment/Salary (full or part-time):; Center for Functional Connectomics, Korea Institute of Science & Technology, Seoul, Korea..

Poster

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Title: Imaging subcellular voltage dynamics *in vivo* with improved genetically encoded indicators

Authors: *F. ST-PIERRE, H. Y. YANG, M. PAN, X. DING, Y. YANG, T. R. CLANDININ, M. Z. LIN;
Stanford Univ., Stanford, CA

Abstract: Nervous systems encode information as spatiotemporal patterns of membrane voltage transients, so accurate measurement of electrical activity has been of long-standing interest. Recent engineering efforts have improved our ability to monitor membrane voltage dynamics using genetically encoded voltage indicators. In comparison with electrophysiological approaches, such indicators can monitor many genetically defined neurons simultaneously; they can also more easily measure voltage changes from subcellular compartments such as axons and dendrites. Compared with genetically encoded calcium indicators, voltage sensors enable a more direct, accurate, and rapid readout of membrane potential changes. However, several challenges remain for *in vivo* voltage imaging with genetically encoded indicators. In particular, current voltage sensors are characterized by insufficient sensitivity, kinetics, and/or brightness to be true optical replacements for electrodes *in vivo*. As a first step towards addressing these challenges, we developed a new voltage indicator, ASAP2, that further improves upon the sensitivity of the fast voltage sensor Accelerated Sensor of Action Potentials 1 (ASAP1). We also describe here how ASAP2 can report stimulus-evoked voltage responses in axonal termini of the fly visual interneuron L2. In this system, ASAP sensors enabled the monitoring of neural activity with greater temporal resolution than three recently reported calcium and voltage sensors. Overall, our study reports novel voltage indicators with improved performance, illustrates the importance of sensor kinetics for accurately reporting neural activity, and suggests L2 as an *in vivo* platform for benchmarking neural activity sensors. We anticipate that ASAP2 will facilitate current and future efforts to understand how neural circuits represent and transform information.

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Poster

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Title: Viral delivery of new biosensors for voltage and cAMP signaling

Authors: S. MARTINKA[1], *T. E. HUGHES[2], P. TEWSON[1], J. PLATISA[3], V. PIERIBONE[3]; [1]MontanaMolecular, Bozeman, MT; [2]Dept Cell Biol/Neurosci, Montana State Univ., Bozeman, MT; [3]The John B. Pierce Lab., New Haven, CT

Abstract: Action potentials are the fundamental currency of neuronal signaling, and concerted activity eventually raises intracellular calcium within the cell. Calcium signaling influences cAMP levels, which in turn cause changes in the activity of a wide variety of proteins and gene transcription. While this chain of events is now doctrine in neuroscience, imaging neural activity is largely limited to biosensors for calcium such as GCaMP6 or GECOs. Here we present fluorescent sensors for voltage and cAMP that can be simultaneously imaged with sensors for calcium in living neurons. For imaging voltage, we used ArcLight, the most robust green fluorescent voltage sensor available today. For cAMP, we created an EPAC-based sensor that is both bright and produces a large change in fluorescence in response to cAMP signaling. Both Arclight and the cAMP sensor were made under the control of the CMV or Synapsin I promoter. These sensors are built with a single green fluorescent protein, making it possible to combine them with red sensors. Both are packaged in a viral delivery system that provides high transfection efficiency, enables transfection of difficult to transfect cell types, including primary cultures, and produces homogeneous cellular expression levels. The genome of the baculovirus used in our delivery system is silent in mammalian cells and produces virus that is non-replicative, making it a biosafety level I reagent. Testing the viruses in HEK 293 cells revealed CMV driven expression of the sensors to be quite consistent from cell to cell. In the case of ArcLight, entire waves of spontaneous depolarization can be captured with remarkable fidelity, and in the case of the cAMP sensor, endogenous Î²2 adrenergic receptor activation produces changes in fluorescence intensity that can be readily detected even with low magnification, low N.A., lenses. In cultures of primary cortical neurons, the CMV version of the Arclight sensor transduced ~25% of the cells in culture (includes glia). The Synapsin I promoter produces restricted sensor expression that is limited to neurons. Pairing each of these green sensors with the red fluorescent R-GECO1.2 calcium sensor makes it possible to simultaneously measure voltage and calcium signaling or calcium and cAMP signaling.

Disclosures: **S. Martinka:** A. Employment/Salary (full or part-time):; MontanaMolecular. **T.E. Hughes:** A. Employment/Salary (full or part-time):; Montana Molecular. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Montana Molecular. **P. Tewson:** A. Employment/Salary (full or part-time):; MontanaMolecular. E. Ownership Interest (stock, stock options, royalty, receipt of

intellectual property rights/patent holder, excluding diversified mutual funds); MontanaMolecular. **J. Platisa:** None. **V. Pieribone:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NS078301

Title: Imaging voltage in brain slices in Cre-lox targeted interneurons with a hybrid voltage sensor

Authors: *P. BAYGUINOV, Y. MA, Y. GAO, X. ZHAO, M. JACKSON;
Dept. of Neurosci., Univ. of Wisconsin, Madison, WI

Abstract: Voltage imaging offers the possibility of studying the concomitant function of large numbers of neurons, and to reveal the emergent properties of neural circuits. Synthetic dyes stain tissues indiscriminately, and in intact circuits the use of these probes is limited to the study of population responses. Recent advances in genetically-encoded voltage probes have allowed voltage imaging in brain slices with single-cell resolution. However, the diversity of neuronal cell types remains an obstacle in exploring the functionality of distinct neural circuits. The development of Cre recombinase driver mouse lines provides a general strategy for targeting probes to specific classes of neurons, allowing the examination of circuits formed by distinct neuronal populations. We have used the Ai35 vector to generate a rosa26 knock-in mouse with a hybrid voltage sensor (hVOS) probe. In the presence of dipicrylamine, this probe serves as a genetically encoded voltage sensor. Cre driver lines for Parvalbumin, glutamic acid decarboxylase 2 and calcitonin-like gene receptor (CalCrl), were crossed with the Ai35-hVOS Cre reporter line to express hVOS probes in three different genetically-defined populations of neurons. Mice from all these crosses express hVOS in sparse populations of neurons in the somatosensory cortex and hippocampus, and report voltage responses to electrical stimulation with single-cell resolution. Voltage imaging experiments revealed action potentials and subthreshold synaptic events in single trial recordings. Furthermore, the generation of this rosa26-hVOS Cre reporter line, generalizes the targeting of hVOS probe to other neuronal subpopulations, depending on the availability of Cre driver lines. This work presents a significant addition to the voltage imaging toolkit, allowing the study of electrical activity in

large groups of specific subpopulations of neurons with high spatial and temporal resolution.
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Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: U01NS090565-01

R01NS083875-02

Title: Semi automated screening system for testing genetically encoded voltage indicators

Authors: *G. VASAN[1,2], J. PLATISA[1,2], A. YANG[1], V. A. PIERIBONE[1,2];
[1]The John B. Pierce Lab., New Haven, CT; [2]Cell. and Mol. Physiol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: In recent years, a number of novel genetically-encoded voltage indicators (GECIs) have been developed. For many of these voltage indicators, including ArcLight - there is an interest to further modify the indicator's sequence (i.e. protein evolution) in order to improve their kinetics and optical response properties. But such a development process demands large-scale mutagenesis of the original probe combined with intense testing of thousands of variants in varied cell systems. Imaging optical response of each variant individually while simultaneously recording electrical response using patch electrodes is a time consuming process. Here, we report on our custom built medium throughput screening process specifically designed to image optical response from cultured neurons in a 96-well plate. The in-house made screening platform is built around a Nikon eclipse Ti microscope equipped with a Perfect Focus system. The movement of the imaging stage, electrode actuator, light source and filter turret rotation are all controlled using custom electronics interfaced with LabView software. The software is programmed to identify the best neurons in each well, stimulate them via a field electrode and record optical response at high temporal resolution (<200 hz) using a Hamamatsu ORCA Flash4.0 camera. Once recorded, a machine vision algorithm identifies and tags neurons in a field of view, performs analysis and

stores response plots in a database to be later reviewed by the researcher. We also discuss the possibilities of using the screening platform for other cell types with varied testing requirements.

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Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: U01 NS090565-01

R01 NS083875-02

Title: Evaluation of genetically encoded voltage indicators (GEVIs) performance in *Drosophila* melanogaster

Authors: *J. PLATISA[1,2], X. JIN[2], M. KUNST[2], M. N. NITABACH[2], V. A. PIERIBONE[1,2]; [1]The John B Pierce Lab., New Haven, CT; [2]Cell. and Mol. Physiol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Neurons exhibit a collection of different transient electrical events that vary in both spatial and temporal domains. To be able to analyze these events *in vivo* we need methods with both high temporal and spatial resolution and large signal to noise (SNR) ratio. Use of light to record brain electrical activity represents a less invasive and more scalable alternative to classical electrode-based methods and provides higher spatial resolution. Optical methods allow simultaneous recordings from different locations within a field of view, from the level of single cell and cell compartments to the level of neuronal circuits comprising of hundreds, even thousands neurons simultaneously. Development of Genetically Encoded Voltage Indicators (GEVIs) whose expression can be cell-type-specific allows for voltage imaging to be preformed in targeted cells and in the structures buried deep within the brain. In the last two decades a range of different voltage indicators have been developed but one of the major problem for their adoption by the wider community is poor and unreliable performance under real world experimental conditions. Indicators often work well in cultures of immortalized cells, less well in neurons *in vitro* and usually not at all in more intact preparations. Here we present a comparative study in which a range of most promising "new generation" GEVIs were subjected to standardized evaluation under a real world experimental paradigm. To this end, we used the

GAL4/UAS binary expression system to develop several transgenic fruit flies (*Drosophila* melanogaster) that express each of the most promising GEVIs in, lateral ventral circadian clock neurons (LNvs). Simultaneous whole-cell current-clamp recordings and high-speed (500Hz) fluorescence imaging allow for precise assessment of GEVIs performance. We compare expression levels, membrane localization, brightness, signal size and SNR of GEVIs under standardized imaging condition and using conventional imaging equipment.

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Poster

733. Technology Development: Projection Mapping

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Program#/Poster#: 733.01/DD5

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Automated dye microinjection to label mouse and human neurons

Authors: *B. R. LONG, E. LEIN, H. PENG;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Neuronal morphology is a key feature for identifying cell types in the brain. In the case of *H. sapiens*, suitable tissue samples are rare and cover a wide range of ages, genetic backgrounds and disease states. To quantitatively assess morphological cell types across such a diverse population, large numbers of digital reconstructions are needed. Although decades of Golgi studies have established morphologically -defined neuronal types across many species, the Golgi method is limited by the its inability to control the precise location of labeled cells. Furthermore, for high-throughput extraction of neuronal reconstructions, the density of labeled cells must be tightly controlled to avoid labeling closely -apposed neurites in neighboring cells. One method that allows control of both location and density of labeled cells for morphological reconstruction is microinjection of fluorescent dye, typically Lucifer Yellow (LY), into the cell body of neurons in lightly fixed samples. In addition to labeling cells in the mouse brain, LY microinjection can be used to target cells in fixed human tissue, allowing many cells to be filled in each slice. However, LY microinjection requires extensive training and an expert manual operator to obtain good quality morphology data. Here we present an automated system for LY microinjection that can be targeted to DAPI-stained neurons in fixed tissue. By reducing LY microinjection to a point-and-click process, we are able to rapidly fill neurons in user-defined regions of fixed slices. Automated cell filling can facilitate high-throughput cell filling targeted

to specific anatomical regions or in a spatially unbiased manner to survey morphologies across the sample.

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Poster

733. Technology Development: Projection Mapping

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Transmembrane transport of proteins into neurones by cholera toxin B

Authors: *J. L. HAIGH[1], W. B. TURNBULL[2], S. DEUCHARS[1], J. DEUCHARS[1];
[1]Fac. of Biol. Sci., [2]Fac. of Mathematics and Physical Sci., Univ. of Leeds, Leeds, United Kingdom

Abstract: Cholera toxin B (CTB), the non-toxic subunit of the toxin produced by Vibrio cholerae is taken up by motor, sensory and autonomic preganglionic neurones in the CNS when administered systemically (Havton & Broman, 2005). This work aims to modify CTB as a means to deliver other proteins into these neurones to influence their function. To test this we chose parvalbumin, which is a calcium buffer protein generally not expressed in motor neurones. A C-terminal fusion protein of CTB and parvalbumin (CTBparv) was expressed via Escherichia coli. Isothermal titration calorimetry revealed that CTBparv maintained binding affinity for its receptor GM1 ganglioside with a Kd = 49 nM, similar to that of wild-type CTB (Kd = 40 nM). This suggested that the fusion protein would be endocytosed, and was confirmed by its uptake by HEK 293T cells after incubation with 5 μg of CTBparv for between 1 and 24 hours. To test if CTBparv is able to be taken up by motor neurones, C57BL6 mice were anaesthetised with isoflurane and 40 μg of CTBparv in 2 μl of 50 mM HEPES administered at multiple sites of the tongue musculature. After 1, 2, 4 or 7 days, mice were anaesthetised with 80 mg/kg pentibarbitol IP and transcardially perfused with 4% paraformaldehyde. The brainstem was sectioned at 50 μm on a vibrating microtome. CTBparv successfully labelled the hypoglossal nucleus as revealed by immunoreactivity to both CTB and parvalbumin. Control mice injected with 40 μg CTB in 2 μl of 50 mM HEPES had CTB labelled hypoglossal neurones with no parvalbumin immunoreactivity. For IP injections, unanaesthetised C57BL6 mice were injected with 2000 μg, 1000 μg, or 500 μg of CTBparv in 100 μL of 50 mM HEPES and after 4 days, tissue was prepared as before. There were CTB immunopositive cells in the dorsal vagal nucleus of the brainstem and motor neurones in the ventral horn of the spinal cord, but not all cells were

positive for parvalbumin. Further experiments will seek to determine the sub-cellular localisation and functionality of the parvalbumin within the neurones. The results highlight CTB as a useful means to deliver other proteins past the blood-brain barrier and into the CNS by entering neurones with axons in the periphery. CTB has the potential to be utilised as therapeutic tool to target specific subsets of neurones within the CNS affected by neurological diseases.

Disclosures: J.L. Haigh: None. **W.B. Turnbull:** None. **S. Deuchars:** None. **J. Deuchars:** None.

Poster

733. Technology Development: Projection Mapping

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 733.03/DD7

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIDA/NIH IRP

Title: Methods for assessing internalization of microparticles into neuronal cells

Authors: *V. WALLACE[1], F. RUBIO[1], R. CIMBRO[2], M. J. HENDERSON[1], L. V. FORTUNO[1], R. MADANGOPAL[1], B. HARVEY[1], B. HOPE[1];
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Abstract: While it has been well-established that neurons can internalize particles on the nano scale, little has been done to assess the internalization of micron-sized particles in neuronal cells. Confirming that neurons can internalize microparticles could open the door to a host of cell-level diagnostic technology. However, few studies have established reliable and widely applicable methods that allow distinction between internalized and surface-adsorbed particles, as well as precise quantification of the internalized fractions. Combining the techniques of flow cytometry, fluorescence activated cell sorting (FACS), epifluorescent and confocal microscopy, we have developed a set of robust and reliable methods for confirming that cells *in vitro* can internalize micron-sized particles. These methods can be applied to a vast array of particle types and sizes, and can allow for further investigation into the characteristics of microparticles that affect uptake into cells. Together, the results will further the development of potential applications of intracellular micron-scale technology in neuronal cells.

Disclosures: V. Wallace: None. **F. Rubio:** None. **R. Cimbro:** None. **M.J. Henderson:** None. **L.V. Fortuno:** None. **R. Madangopal:** None. **B. Harvey:** None. **B. Hope:** None.

Poster

733. Technology Development: Projection Mapping

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant 5R01NS073129-05

NIH Grant 5R21DA035538-02

11233/ALLEN

Title: SHAFT: A novel method for mapping long-range projections at single neuron resolution and in high-throughput using DNA sequencing

Authors: *J. M. KEBSCHULL[1], P. GARCIA DA SILVA[2,1], I. PEIKON[1], D. F. ALBEANU[1], A. M. ZADOR[1];
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Abstract: Long-range connections provide the substrate for integrating information across brain areas. Recently, several large-scale efforts, such as the Allen Mouse Brain Connectivity Atlas, have provided a systematic survey of long-range connectivity in the mouse brain. However, although knowledge of the aggregate connectivity from one brain area to another has proven invaluable, in some cases resolution at the circuit and even single neuron level is needed. Unfortunately, established methods for determining projection patterns at higher resolution are extremely labor intensive and therefore low-throughput. Here we describe SHAFT ("Sequence-based High-throughput Analysis of Far Targets"), a relatively simple, inexpensive, and highly muti-plexed method for establishing the projection pattern of single neurons in the mouse brain. Our method transforms the problem of long-range connectivity from a problem of microscopy into a problem of DNA sequencing, thereby enabling us to exploit the tremendous recent increase in sequencing throughput. In our approach, we label a defined population of neurons using a viral tracer injection. However, instead of labeling the population with a fluorophore such as GFP, we use viruses to express short random sequences of RNA or "barcodes". A barcode of length 30 nucleotides has a theoretical diversity of 4^30=10^18, far more than the number of neurons in the mouse brain (~10^8). Random barcodes can thereby provide a unique label for all infected neurons. We engineered the RNA barcodes to bind to a modified pre-synaptic protein, so that upon co-expression, the protein drags the RNA barcode to distal axonal projections. We then use high-throughput DNA sequencing to read out projection patterns at

selected regions of interest (ROIs). By determining which barcodes are present at which ROIs, we obtain the projection patterns of single neurons. Our method scales well, and has the potential to provide a systematic survey of long-range projections in the mouse brain at single neuron resolution.

Disclosures: J.M. Kebschull: None. **P. Garcia da Silva:** None. **I. Peikon:** None. **D.F. Albeanu:** None. **A.M. Zador:** None.

Poster

733. Technology Development: Projection Mapping

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NIMH IRP project MH002497-24

Title: Neocortical projection neurons receive class-specific patterns of long-range inputs

Authors: *K. E. BORGES[1], N. YAMAWAKI[1], I. R. WICKERSHAM[2], C. R. GERFEN[3], G. M. G. SHEPHERD[1];
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Abstract: Excitatory cells in the cerebral cortex can be classified based on their axonal projections. Intratelencephalic (IT) neurons terminate exclusively within cortex and striatum, and are the only cortical neurons that project contralaterally. Pyramidal tract (PT) neurons send axons toward the brainstem and spinal cord and branch into ipsilateral cortex, striatum, thalamus, and midbrain. Finally, corticothalamic (CT) neurons project to the thalamus. Each cortical area contains its complement of IT, PT, and CT neurons. However it is not known, in a given brain area, whether these three projection classes receive input from the same, partially overlapping, or entirely separate areas of the brain. We pursued this question using viral methods for

monosynaptic tracing, focusing on primary motor cortex and (in separate experiments) auditory cortex of the mouse. We used mice from BAC-Cre driver lines that express Cre recombinase in IT (line Sepw1_NP39 for layer 2/3 and line Tlx3_PL56 for layer 5), PT (line Sim1_KJ18), or CT (line Ntsr1_GN220) neurons. We injected either motor or auditory cortex with adeno-associated virus (AAV) encoding cre-dependent TVA (the receptor for EnvA) and rabies virus glycoprotein. The same cortical area was later injected with glycoprotein-deleted rabies virus encoding a red fluorescent protein and coated with EnvA. Complementation of glycoprotein by AAV allowed rabies virus to retrogradely jump across one synapse, labeling the monosynaptic inputs to either PT, IT, or CT neurons. Preliminary findings indicate that IT, PT, and CT neurons receive long-range connections from distinct but partially overlapping cortical and subcortical areas. For example, in motor cortex all three classes receive input from retrosplenial cortex, but IT and CT neurons tend to receive more input from premotor and orbital areas than do PT neurons, and only PT and layer 2/3 IT neurons receive input from secondary somatosensory cortex (S2). All three classes receive input from thalamus, but from somewhat different subsets of thalamic nuclei. IT and CT neurons receive input from globus pallidus, whereas PT neurons do not. Similarly, projection classes in auditory cortex receive differential input from frontal, motor, and somatosensory cortical areas, as well as from the medial geniculate body and other subcortical areas. Our results suggest that IT, PT, and CT neurons may route information from different subsets of presynaptic sources to their respective targets throughout the brain.

Disclosures: **K.E. Borges:** None. **N. Yamawaki:** None. **I.R. Wickersham:** None. **C.R. Gerfen:** None. **G.M.G. Shepherd:** None.

Poster

733. Technology Development: Projection Mapping

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH AG010435

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Veterans Administration

Title: C.O.M.E.T: Optimized genetic tracers for viral mediated neuronal projection mapping

Authors: J. N. DULIN[1], E. VAN NIEKERK[1], T. GRIDER[1], M. H. TUSZYNSKI[1,2], *D. GIBBS[1];
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Abstract: Existing neuronal tracing techniques have greatly facilitated the detailed study of the anatomy and connectivity of neurons in the nervous system. Mapping of axonal projections using these tracing techniques has allowed investigators to study how neural circuits may change in response to the environment, during development or in disease states. Viral vectors, in particular rAAV-based vectors, provide the ideal vehicle for delivery of genetically encoded neural tracers to specific neuronal populations. Any enhancement in the resolution of existing neuronal tracers will potentially lead to new insights into neuronal anatomy and connectivity in the intact or damaged nervous system. To improve the detection sensitivity and spatial resolution of genetically encoded tracers, we have developed AAV8 vectors expressing Codon Optimized Membrane Embedded Tracers (COMET). Green fluorescent (gCOMET) and red fluorescent (rCOMET) tracers were derived from the highly fluorescent superfolderGFP and tdTomato fluorescent proteins respectively. The sensitivity and spatial resolution of axonal projections traced using AAV8-COMET was quantified in the adult rat spinal cord and optic nerve following vector delivery to either corticospinal motor neurons (CST) or retinal ganglion cells (RGC). AAV8-COMET traced axons were compared to axons traced using either titer-matched AAV8-EGFP or Biotinylated Dextran Amine (BDA). In both the spinal cord and optic nerve, a significant increase in the detection of axons was observed using AAV8-COMET when compared to AAV8-EGFP. AAV8-COMET tracing also resulted in markedly improved spatial sensitivity, with a significant increase in the number and density of labeled fine axonal processes detected. The increased sensitivity and resolution with AAV8-COMET was even more dramatic when compared to BDA tracing in the same animals, highlighting the limitations of this commonly used chemical tracing technique. AAV8-COMET tracing of the CST in rat spinal cord identified novel patterns of CST innervation. In addition, AAV8-COMET tracing of CST in a rat model of axonal regeneration following spinal cord injury (Lu et al, Cell 2012) demonstrated dramatically increased detection of regenerating axons penetrating a neural stem cell graft at the site of injury. These studies demonstrate the utility of using COMET vectors to uncover previously undetectable neuronal anatomy and connectivity patterns in the intact or diseased nervous system.

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Poster

733. Technology Development: Projection Mapping

Location: Hall A

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Program#/Poster#: 733.07/DD11

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSC 102-2311-B-002-034-MY3

Title: Reversed topographical projection pattern from medial thalamus to anterior cingulate cortex

Authors: *H.-Y. YEH[1], I.-C. WU[1], J.-C. LEE[1], C.-T. YEN[1,2];
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Abstract: Thalamocortical connections between medial thalamus and cingulate cortex are important for emotional and cognitive functions. Functional imaging studies of the anterior cingulate cortex revealed anterior-posterior subdivisions. It is, therefore, interesting to test whether there is a rostrocaudal differentiation of connection between medial thalamus and anterior cingulate cortex. We tested this hypothesis by injecting the retrograde tracer, FluoroGold (FG), into the cingulate cortex of the rat from 4 mm rostral (A4) to 1 mm caudal to the bregma and analyzed the distribution of the anti-FG immunohistochemically positive thalamic neurons quantitatively. A tendency of reversely connected pattern from medial thalamus to anterior cingulate cortex was observed. Rostral part of the anterior cingulate cortex received heavier projections from the caudal part of the medial thalamus; and conversely, the caudal part of the anterior cingulate cortex received more projections from rostral medial thalamus. These medial thalamic nuclei included mediodorsal nuclei, paraventricular thalamic nuclei, ventromedial nucleus, intralaminar nuclei and the midline nuclei. Cingulate cortex posterior to A1 had lighter projection from medial thalamus, but received most of their thalamocortical projection from the anterior thalamic nuclei. This topographically arranged connection with the medial thalamus may underlie the functional heterogeneity in the anterior cingulate cortex.

Disclosures: H. Yeh: None. **I. Wu:** None. **J. Lee:** None. **C. Yen:** None.

Poster

733. Technology Development: Projection Mapping

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NINDS R01NS081040

R21NS082835

The Miami Project to Cure Paralysis

Buoniconti Fund

Title: 3d imaging of axons in transparent spinal cords from rodents and nonhuman primates

Authors: *D. LEE, C. SODERBLOM, A. DAWOOD, M. CARBALLOSA, A. J. SANTAMARIA, F. D. BENAVIDES, S. JERGOVA, R. M. GRUMBLES, C. K. THOMAS, K. K. PARK, J. D. GUEST, V. P. LEMMON, J. LEE, P. TSOULFAS;
Univ. of Miami Miller School/ Miami Project, Miami, FL

Abstract: The use of anterograde and retrograde dyes as well as genetic labeling techniques has advanced our understanding of the structure-function relationship of the CNS. Most of this of data is collected from two dimensional histological sections, which makes it difficult to accurately study complex anatomical and structural changes that occur during development and disease. Therefore, three dimensional visualization and quantification directly from whole CNS tissue would immensely benefit studies on CNS structure and function. The histological assessment of spinal cord tissue in three dimensions has previously been very time consuming and prone to errors of interpretation. Advances in tissue clearing have significantly improved visualization of axonal origins and targets. While these proof-of-concept studies have been performed with transgenic mice in which axons were pre-labeled with green fluorescent protein (GFP), investigating axonal paths and regeneration often requires stringent axonal tracing methods. Using mouse and rat models of spinal cord injury, we labeled different axon tracts using several types of adeno-associated viruses (AAVs) and chemical-based tracers and performed tetrahydrofuran-based tissue clearing to image multiple axon types in the spinal cord using light sheet and confocal microscopy. Using this approach, we investigated the relationships between axons and scar-forming cells at the injury site as well as connections between sensory axons and motor pools in the spinal cord. In addition, we used these methods to trace axons in nonhuman primates. This reproducible and adaptable virus-based approach can be combined with transgenic mice or with chemical-based tract-tracing methods, providing scientists with flexibility in obtaining axonal trajectory information from transparent tissue.

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Poster

733. Technology Development: Projection Mapping

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: ANMS Research Award 2014

Title: Labeling of vagal terminals in the area postrema of Nav1.8-Cre-ChR2 mice

Authors: *L. GAUTRON;
Univ. Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Neurons located in the area postrema exclusively receive synaptic input from vagal afferents. In this study, Nav1.8-Cre-ChR2 mice with restricted expression of the Channelrhodopsin 2 (ChR2) in Nav1.8-expressing afferents were used to fluorescently label vagal terminals in the area postrema. In these mice, the membrane of nodose ganglion neurons and their axons terminating in the dorsovagal complex including the area postrema were marked with bright green fluorescence. Using confocal microscopy and immunohistochemistry, we identified ChR2-labeled specialized endings of vagal origin resembling synapse-like contacts. In summary, Nav1.8-Cre-ChR2 mice can be useful for further studies of vagal terminals in the area postrema.

Disclosures: L. Gautron: None.

Poster

733. Technology Development: Projection Mapping

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH AG10124

NIH NS053488

Michael J. Fox Foundation

NIHM T32 MH14654

Title: Development of fluorescent small molecules capable of labeling Lewy body pathology in Parkinson's disease tissue

Authors: *T. J. GRAHAM[1], M. BJERKE[2], P. T. KOTZBAUER[3], V. M. -. LEE[2], J. Q. TROJANOWSKI[2], R. H. MACH[1];
[1]Radiology, [2]Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA; [3]Neurol., Washington Univ., St. Louis, MO

Abstract: Background Dementia with Lewy bodies (DLB) and Parkinson's disease with dementia (PDD) are progressive neurodegenerative diseases characterized by intracellular neuronal accumulations in the brain, termed Lewy bodies, composed of a protein called α-synuclein. A large portion of patients afflicted with DLB or PDD also show the pathological hallmarks (Aβ plaques and tau tangles) of Alzheimer's disease (AD) at autopsy. So far, no tracer or fluid biomarker has proven to reliably detect Lewy body pathology *in vivo*. Monitoring of early regional disease progression through *in vivo* visualization of Lewy body pathology through positron emission tomography (PET) imaging would improve the understanding α-synuclein pathological mechanisms and early accurate diagnosis, which will have profound implications on early treatment. **Methods** The affinity of tg-1-25 and similar compounds for aggregated Aβ, tau, α-synuclein fibrils was determined using fluorescence based thioflavin-T displacement assay. Immunohistochemistry (IHC) was performed by incubating the autofluorescent ligand (emission λ ~540 nm) on fresh frozen human PDD and AD tissue. Double labelling (with tg-1-25 and antibodies directed to detect Lewy bodies, Aβ plaques and tangles) was performed to verify ligand binding to specific target pathology. The primary antibodies were coupled to secondary fluorescent antibodies (emission λ 618 nm) and mounting medium containing DAPI (emission λ 460 nm) in order to visualize intracellular and target co-localization of tg-1-25 by fluorescent microscopy. **Results** A high throughput screen identified alkylidene-aurones as binding agents to aggregated α-synuclein fibrils. Subsequent structure modification improved affinity for α-synuclein fibrils (<20 nM; >100 nM for Aβ fibrils) and resulted in the discovery of tg-1-25. It was observed that aurone dienes, such as tg-1-25 exhibited moderate autofluorescence, and could thus be utilized for IHC. Tg-1-25 is capable of staining Lewy body pathology in PDD human tissue, but also stains extracellular Aβ plaques and to some extent intracellular tangles in AD tissue at the concentration needed for IHC. Radiolabeling of tg-1-25 with Fluorine-18 may provide suitable agents for the detection of Lewy Body pathology. **Conclusions** Initial optimization of alkylidene-aurones through structure modification produced tg-1-25, an autofluoresent small molecule that binds α-synuclein and Lewy body pathology in PDD human

tissue. Selectivity over extracellular amyloid plaques and intracellular tangles was not observed, although this may be the result of high ligand concentration required for IHC.

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Poster

733. Technology Development: Projection Mapping

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Program#/Poster#: 733.11/DD15

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIDCD R03 DC10245

Title: The use of optogenetic vector constructs as a dynamic neuronal tracer

Authors: *D. C. PETERSON, G. MLYNARCZYK;
Biomed Sci., Iowa State Univ., Ames, IA

Abstract: Optogenetic vector constructs provide a novel technique that allows reversible light activation or deactivation of individual brain circuits. Although these techniques are promising, the transport activity of the individual vector constructs has never been documented. Because the analysis of optogenetic experiments could vary dramatically based on whether the vector constructs are transported anterogradely, retrogradely, or trans-synaptically; the transport activity for each vector construct must be documented. The results describe the transport of the AAV5-CaMKIIa-eNpHR 3.0-EYFP vector construct obtained from the stock stored at UNC Vector Core at Chapel Hill, NC. Injection into the amygdala showed both terminal and neuronal labeling in areas known to project to and receive projections from the amygdala, however no trans-synaptic labeling was observed. These results indicate that the vector is transported readily in both anterograde and retrograde directions. Because the full pathway seems to be labeled, it is possible that light may activate the light-sensitive chloride channels at any portion of the pathway. Therefore care must be taken when analyzing the results of light activation so that the results reflect all pathways that could be influenced by the light.

Disclosures: D.C. Peterson: None. **G. Mlynarczyk:** None.

Poster

733. Technology Development: Projection Mapping

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 733.12/DD16

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: *In vivo* tracing of neurons using dye delivery electrodes

Authors: *S. L. HEIZMANN, A. KILIAS, P. RUTHER, U. EGERT, M. ASPLUND;
Dept. of Microsystems Engin., Univ. Freiburg, Freiburg, Germany

Abstract: Introduction: A stable long term monitoring of neuronal signals is essential for the investigation of disorders like epilepsy and Parkinson's disease. Penetrating microelectrode probes can be inserted in mouse hippocampus and measure local field potentials (LFPs) revealing information about neural network activities in diseased animals. Histological analysis provides additional information about the cell type and status. To correlate LFP recordings with the underlying histology, a precise labelling of neurons *in vivo* is necessary. However there is a lack of suitable techniques. Therefore a new approach was developed to pinpoint the exact electrode positions in neuronal tissue, using a combined PEDOT/dye system. A conductive PEDOT/PSS coating on top of the recording electrodes allows a controlled exchange of positively charged neurotracer dyes. Defined dye release can thus be triggered at the experimental endpoint after weeks of implantation and limited to the neurons present at the electrode site. Methods: A PEDOT/PSS coating was electrochemically grown on top of iridium oxide (IrOx) recording electrodes integrated in single shaft multielectrode probes. Positively charged dye DiI was incorporated into the PEDOT/PSS layer using an ion exchange method. The coated single shaft multielectrode probe was implanted into mouse hippocampus for LFP recordings. Two weeks after implantation the electrodes were triggered to release the dye. Probes were explanted, the tissue sectioned and the neuronal staining confirmed by fluorescence microscopy. Results: The proof of concept measurements with spectrofluorometry *in vitro* show the possibility to exchange the neurotracer dye DiI in a PEDOT/dye coating in well controllable mode. A reduced impedance and stable LFP recordings *in vivo* show the suitability of this approach for recording electrodes. The PEDOT/PSS layer was tested to act as dye reservoir for time periods of weeks. Subsequently the dye can be actively released by electroactivation of the PEDOT/dye system. The released dye locally stained the neurons and allowed a retroactive tracing of electrode positions in hippocampal tissue. Correlation of cell labelling with the probe shaft lesion site was evaluated.

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Poster

734. Whole-Brain Imaging and Atlasing II

Location: Hall A

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Program#/Poster#: 734.01/DD17

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: ONR Grant N000141210214

SIU School of Medicine

Title: A digital rat brain atlas derived from ultra-high-resolution MRI images scanned in three dimensions

Authors: ***T. J. BROZOSKI**[1], B. ODINTSOV[2], D. T. BROZOSKI[1], K. W. WISNER[1]; [1]Div. Otolaryngology, SIU Sch. of Med., Springfield, IL; [2]Beckman Imaging Ctr., Univ. of Illinois, Urbana Champaign, Urbana, IL

Abstract: Objective. To derive an idiographic high resolution brain atlas from *in situ* MRI images of adult Long Evans rats. Subjects. Two young adult Long Evans (Harlan, Indianapolis, IN, USA) male rats, wt. 506 - 510 g age 135 days, were imaged under identical conditions on successive days. The experimental protocol was approved by the Laboratory Animal Care and Use Committee of Southern Illinois University School of Medicine. Imaging. Magnetic resonance images were acquired on an ultra-high resolution micro-imaging scanner (Oxford Instruments, Abington, UK) equipped with a Unity/Inova console (Varian, Palo Alto, CA), operating at 14.1 T. Image slices were 0.2 mm thick, with a planar resolution of 100 µm, and were acquired using 20 scans per slice. Images were processed using custom-written Matlab codes that reduced contrast variation between slices and improved image quality. A patented tunable transmit/receive radiofrequency (RF) coil was used for image acquisition (Odintsov, 2011). Obtained from each brain were 120 transverse (coronal), 70 saggital, and 48 horizontal slices. Subject preparation. Scans were acquired *in situ* and *ex vivo*. Immediately before imaging the rats were lethally anesthetized (Euthasol, Virbac, Ft. Worth, TX), decapitated, and their dorsal skull exposed. Holes were drilled into the skull at Bregma and Lambda (defined in Paxinos and Watson, 1998) and each was filled with gelfoam saturated with 3 mM CuSo4. These provided empirical image landmarks at Bregma and Lambda. Image reconstruction. TIFF images of each brain slice, produced by MatLab code, were imported into Adobe Photoshop (CS2 v9.0, Adobe Systems) and saved in Photoshop format at 500 pixels/inch. Photoshop was used to enhance image contrast, rotate to a normal orientation, and overlay a 1 mm grid. Image dimensions were unaltered. Descriptive labels were added to each image and surrounding tissue

was digitally removed, except where tissue landmarks were useful. Image brightness and contrast were normalized to a standard. Atlas assembly and viewing. Images, one slice per page, were assembled into a PDF book, of 3 chapters, each devoted to a different slice orientation (transverse, saggital, horizontal). The atlas can be viewed using Adobe Reader. Each image consists of 4 layers (named below) that can be independently turned on or off. “Brain” depicts the brain and its associated labels (Bregma/Lambda coordinates, etc.). “Grid” depicts color-contrasted rectilinear gridlines. “Reference Lines” indexes the dorsal surface of cortex, midline in transverse sections, and Bregma and Lambda in transverse and saggital sections. “Background” enables a black background to be turned on and off.

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Poster

734. Whole-Brain Imaging and Atlasing II

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Program#/Poster#: 734.02/DD18

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSF IDBR:1354015

Title: Development of an SLM-based sheet illumination microscope for large-scale 3-D neural structure and dynamics observation in model organisms

Authors: ***B. A. MADRUGA**[1,2], L. A. BENTOLILA[3,2], K. ARISAKA[1,2];
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Abstract: Sheet Illumination microscopy has made a large impact on the microscopy community due to its many advantages. Increased photon efficiency allows for lower power light sources, which in turn reduce phototoxic damage to the sample, while providing an increased signal to noise ratio. To take advantage of such technique, a type of phase modulator, known as a Spatial Light Modulator (SLM) is used to generate a deep-penetrating, extremely long and narrow bessel beam pattern. Through the use of an SLM, one can easily modulate characteristics of the illuminative beam in real time, to generate multiple versions and combinations of Gaussian beams, sectioned bessel beams, and lattice beams for example- enabling greater flexibility and faster readout speed, while ensuring high resolution across multiple scientific applications. The bessel beam provides a much longer region of micron-order uniformity along the illumination

axis, mapping well onto the rolling-shutter readout of the sCMOS camera, resulting in orders of magnitude improvement in data acquisition speed with line-confocal resolution. A piezoelectric objective collar is used along the detective axis, to enable rapid z-dimensional scanning in depth, thereby creating three-dimensional volumes with adequate time resolution to characterize and observe active neural dynamics in systems of several hundred neurons across multiple species, such as zebrafish and C.Elegans. Long working distance, refractively-corrected detective objectives, specifically designed for the observation of clarified tissue, such as CLARITY-treated neural tissue, along multiple wavelength channels are used for studying neurobiology on a whole mouse brain scale in multiple magnifications. Utilizing several high speed, sub-micron accuracy linear stages, it is possible to take many data sets quickly and continuously, to acquire three dimensional information on the order of 1.5 cm cubed, with sub-micron spatial resolution in a matter of hours. Tools of such flexibility will enable the study of large-scale neuronal activity and structure under controlled or experimental conditions across many model organisms.

Disclosures: **B.A. Madruga:** A. Employment/Salary (full or part-time):; UCLA. **L.A. Bentolila:** None. **K. Arisaka:** A. Employment/Salary (full or part-time):; UCLA.

Poster

734. Whole-Brain Imaging and Atlasing II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSF IGERT Fellowship

Kavli Foundation

MURI W911NF-12-1-0594

Wallace H. Coulter Foundation

NSF 0954796

R01NS063226 (NINDS)

R01 NS076628 (NINDS)

Title: Swept confocally aligned planar excitation (scape) microscopy for large-scale brain imaging in adult *Drosophila* melanogaster

Authors: *W. LI[1], V. VOLETI[2], E. S. SCHAFFER[3], C. MENDES[4], N. MISHRA[3], R. S. MANN[4], E. M. C. HILLMAN[2];
[2]Biomed. Engin., [3]Neurosci., [1]Columbia Univ., NEW YORK, NY; [4]Biochem. and Mol. Biophysics, Columbia Univ. Med. Ctr., NEW YORK, NY

Abstract: Understanding complex, distributed neural circuits depends heavily on our ability to observe them in action. Although the size of the adult *Drosophila Melanogaster* brain makes it accessible to light microscopy, standard confocal and two-photon approaches are limited in their ability to achieve sufficiently high volumetric imaging rates over large enough fields of view to capture whole-brain activity. Here, we use Swept Confocally Aligned Planar Excitation (SCAPE) microscopy to image brain activity of adult behaving *Drosophila*. SCAPE is a translationless, single-objective light sheet imaging technology recently developed by our group, which enables single-cell resolution recording at over 10 volumes per second with large fields of view. Compared with conventional dual-objective light sheet configurations, the single objective configuration of SCAPE makes it more suitable for *in vivo* brain imaging in awake, behaving animals. In combination with diverse genetic tools, SCAPE's temporal and spatial capabilities make it an ideal match for studying the complexity of neural processing in the *Drosophila* brain. We have used SCAPE to record multi-region brain activity in response to olfactory stimuli in GCaMP6-labelled, behaving adult fruit flies. We are currently utilizing more specific labeling strategies to permit more complex analysis of olfactory circuits throughout the brain, and establishing the ability to simultaneously track and alter walking behavior. Our latest results will be presented.

Disclosures: W. Li: None. **V. Voleti:** None. **E.S. Schaffer:** None. **C. Mendes:** None. **N. Mishra:** None. **R.S. Mann:** None. **E.M.C. Hillman:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSF EBICS 0939511

Title: 3d murine brain connectome reconstruction using spatial light interference microscopy (slim)

Authors: *S. KIM[1], E. MIN[1,2], L. MA[1,3], W. JUNG[2,4], G. POPESCU[1], C. BEST-POPESCU[1]; [1]Bioengineering, Univ. of Illinois At Urbana Champaign, Urbana, IL; [2]Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of; [3]Zhejiang Normal Univ., Jinhua, China; [4]Ctr. for Soft and Living Matter, Inst. of Basic Sci., Ulsan, Korea, Republic of

Abstract: Mapping neural networks is important in studying cerebral development and disease states of the brain. Recently, the use of fluorescence in combination with various methods of tissue clearing, have revealed entire mouse brain connectivity. However, when using fluorophores, the information gathered is limited to only specific structures tagged in the brain. Here we present a holistic picture of the brain microstructure using label-free imaging. We visualized distributed neuronal circuits in mouse brain slices using Spatial Light Interference Microscopy (SLIM). SLIM is a novel microscopy technique that provides quantitative phase imaging information with nanoscale sensitivity to pathlength and high-transverse resolution. We then reconstructed the three dimensional neural circuit topology through image mosaicking. We demonstrate the successful reconstruction of a group of neuronal networks with subcellular resolution. With SLIM, we are able to image long-range and local connections, and reveal the macro architecture of neural networks as well as single cell cartography. This novel murine connectome visualization technique provides the opportunity for studying neural connections in both healthy and disease brain models.

Disclosures: S. Kim: None. **E. Min:** None. **L. Ma:** None. **W. Jung:** None. **G. Popescu:** None. **C. Best-Popescu:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 734.05/DD21

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Neuron reconstruction for allen cell types database

Authors: *Z. ZHOU[1], X. LIU[2], S. SORENSEN[2], M. FISHER[2], D. SANDMAN[2], A. HENRY[2], N. THATRA[2], T. DESTA[2], W. WAKEMAN[2], S. SUNKIN[2], E. LEIN[2], H. ZENG[2], M. HAWRYLYCZ[2], J. PHILLIPS[2], C. KOCH[2], H. PENG[2];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: 3D neuron morphology reconstruction at single-neuron resolution at large scale remains a challenging task in neuroscience. Accurate neuron reconstructions are crucial for

morphology quantification and cell type classification. For the Allen Cell Types Database at the Allen Institute for Brain Science, 3D neuron morphologies are reconstructed from the biocytin labeled bright-field large-scale mouse neuron images, which present the following two challenges: 1) Unlike confocal images, bright-field images contain a substantial amount of noise and have high intensity backgrounds; and 2) The size of the image data is typically in the range of 10 to 100 GB, which is too large to process using standard PCs. We present our neuron reconstruction workflow for the Allen Cell Types Database project. First an adaptive image enhancement method was used to improve the signal-to-noise ratio of bright-field images by detecting the salient features of neuron structures with adaptive estimation of the optimal window size. We then deployed an automatic 3D neuron tracing method called NeuronCrawler to efficiently reconstruct large-scale neuron images by iterating a depth-first search strategy over a number of image tiles with continuous neuron structures. Finally, manual curations were carried out on the auto-traced results to finalize the 3D neuron morphologies by applying a family of new human-machine interaction Virtual Finger algorithms. We used the open source software Vaa3D (http://vaa3d.org) for automatic reconstructions and manual annotations, as well as visualizations (Figure 1).

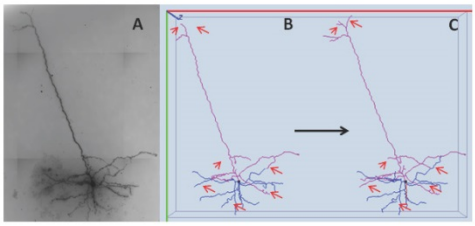

Figure 1. Example image of a single biocytin-filled neuron and its associated uncorrected and corrected autotrace. A. Minimum intensity projection image of a biocytin-filled neuron. B. 3D automated reconstruction. C. Final reconstruction following manual correction. (Dark pink = Apical dendrite, blue = dendrite) It took approximately 3.5 hours to manually correct the automated reconstruction.

Disclosures: **Z. Zhou:** None. **X. Liu:** None. **S. Sorensen:** None. **M. Fisher:** None. **D. Sandman:** None. **A. Henry:** None. **N. Thatra:** None. **T. Desta:** None. **W. Wakeman:** None. **S. Sunkin:** None. **E. Lein:** None. **H. Zeng:** None. **M. Hawrylycz:** None. **J. Phillips:** None. **C. Koch:** None. **H. Peng:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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Program#/Poster#: 734.06/DD22

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant 1R03NS077295-01

Title: A multi-modal atlas of the zebrafish brain

Authors: *J. F. ULLMANN[1], A. L. JANKE[2], N. KURNIAWAN[2], M. WULLIMANN[3], D. REUTENS[2];
[1]The Univ. of Queensland, St. Lucia, Australia; [2]The Univ. of Queensland, Brisbane, Australia; [3]Ludwig Maximilians- Univ., Planegg-Martinsried, Germany

Abstract: The zebrafish is a premier model in neuroscience research. As vertebrates they share significant physiological and genetic homology with humans. They possess the vertebrate brain archetype as well as all major neurotransmitters, hormones and receptors. While the larval zebrafish has been traditionally used, more recently experiments with adult zebrafish are becoming widespread due their well-developed sensory, motor, and endocrine systems, and diverse behaviour. This has been demonstrated by the use of adult zebrafish as an important model for a range of neurological diseases such as epilepsy, Parkinson's disease, autism, post-traumatic stress disorder, and addiction. Despite the increased use of the model, few detailed maps of the adult zebrafish brain exist. Therefore we have developed a detailed multi-modal atlas of the zebrafish brain. The atlas is made up of 1) a minimum deformation model generated from 23 high-resolution magnetic resonance imaging data sets (AB strain); 2) five super-resolution short-track density imaging data sets; and 3) six brains cleared and labelled for myelin, dopamine, serotonin, acetylcholine, γ-Aminobutyric acid, and parvalbumin. This data has been nonlinearly registered into a single template space and segmented into just under 150 structures. We have also created minimum deformation models for the Absolute, Tübingen and Tübingen long fin strains and performed voxel-based morphometry to compare differences in brain structure. The complete data set will be a freely available reference atlas and resource for automatic segmentation of novel zebrafish mutants.

Disclosures: J.F. Ullmann: None. **A.L. Janke:** None. **N. Kurniawan:** None. **M. Wullimann:** None. **D. Reutens:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSF CBET 1344219

NIH 1DP1NS087724

Title: Expansion microscopy of lipids for scalable morphological analysis of neurons and neural circuits

Authors: *E. D. KARAGIANNIS, A. H. MARBLESTONE, E. S. BOYDEN;
MIT, Cambridge, MA

Abstract: Although electron microscopy provides a platform to image and reconstruct neuronal morphology with nanoscale resolution, it is slow, expensive, does not easily facilitate multicolor labeling, and is not readily accessible to most neuroscientists. Recently, our lab has introduced a new modality of imaging, which uses physical sample magnification, Expansion Microscopy (ExM; Science 347(6221):543-548), which allows imaging of large 3D specimens with nanoscale resolution because fast diffraction-limited optical microscopes can be used. The physical magnification of the sample results from synthesizing a polyelectrolyte gel network directly within the specimen, which is then physically expanded to achieve a ~50nm resolution using a common confocal microscope. Applying ExM to the imaging of neuronal membrane morphology requires the development of a lipid-binding tag which can be anchored to the ExM hydrogel. We have now designed and implemented ExM-compatible lipid-binding labels that can selectively bind the plasma membranes of neurons and other cells. These labels are water soluble, and have small molecular weights (<2kDa), which facilitates the fast 3D diffusion of the labels within tissue, and thus allowing staining of large tissue volumes. The tags contain chemical groups that facilitate the chemoselective conjugation of multiple types of fluorescent labels, important for multicolor and amplified labeling. These chemical approaches will allow ExM-based analysis of neural ultrastructure in diverse neural circuits, including that of the human brain.

Disclosures: E.D. Karagiannis: None. **A.H. Marblestone:** None. **E.S. Boyden:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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Support: Allen Institute for Brain Science

MIT Media Lab and MIT McGovern Institute

NIH 1R01EY023173

MIT Synthetic Intelligence Project

IET Harvey Prize

NSF CBET 1053233

New York Stem Cell Foundation-Robertson Award

Title: Sparse reconstruction light-field microscopy for high-resolution 3d-imaging of neuronal activity

Authors: *Y.-G. YOON[1], N. PAK[2], L. FREIFELD[3], M. A. HENNINGER[4], J. DEGUCHI[3], N. SAVIDIS[3], E. S. BOYDEN[5];
[1]Dept. of Electrical Engin. and Computer Sci., [2]Dept. of Mechanical Engin., [3]Media Lab., [4]Dept. of Physics, [5]Media Lab. and McGovern Institute, Departments of Biol. Engin. and Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: We recently developed an approach using light-field microscopy (LFM) to image the entire *C. elegans* nervous system and the entire zebrafish brain in 3D, at high speeds (e.g., ~20 Hz), possible since no moving parts are required for light-field microscopes to acquire 3-D images (Prevedel et al., 2014). However, the spatial resolution was limited, and the activity of only a subset of the neurons could be extracted as it relied on a computational method based on independent component analysis (ICA) to extract the activity of neurons during post-processing. For ground-truth studies of the nervous system, ideally it would be possible to pick up the activity of all neurons, even those that are quiet during the recording (whereas ICA tends to select for highly active neurons). And, ideally, it would be possible to assign neural activity to defined sites in the circuit, in order to link anatomical and dynamical descriptions of neural circuits. We have developed a novel sparse reconstruction lightfield microscopy (SRLFM) strategy that can, in simulation, accurately reconstruct neural activity throughout the entire brain of the larval zebrafish, expressing a GCaMP variant, with single cell resolution, at up to 100 Hz. No post-processing (e.g., via ICA) is needed with SRLFM, meaning that even quiet neurons can

be detected, and also neural activity can be accurately assigned to specific neurons in the neural network, important for linking neural circuit dynamics to the underlying network architecture. The effective resolution of light-field microscopy is improved by >2-fold with our new algorithm. We are currently implementing this algorithm on an optimized light-field microscope, aiming to assess the technology in the context of living brain dynamics. (Yoon, Pak and Freifeld are co-first authors.)

Disclosures: Y. Yoon: None. **N. Pak:** None. **L. Freifeld:** None. **M.A. Henninger:** None. **J. Deguchi:** None. **N. Savidis:** None. **E.S. Boyden:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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Support: NIH 1DP1NS087724

NIH 1R01MH103910-01

NSF CCF-1231216

NSF CBET 1053233

Jeremy and Joyce Wertheimer

NSF GFRP

Fannie and John Hertz Foundation

Title: Nanoscale resolution, multiplexed, biomolecular imaging of brain circuits via expansion microscopy

Authors: *F. CHEN, A. T. WASSIE, S. ALON, E. S. BOYDEN;
MIT, Cambridge, MA

Abstract: Nanoscale-resolution imaging of RNA transcripts and proteins throughout brain circuits in a multiplexed fashion would enable the molecular characterization of cell types, synapses, and signaling pathways, in normal as well as pathological brain states. Although many multiplexing strategies, primarily using serial application of affinity tags and/or biomolecule-

encoding nucleic acid barcodes, have been demonstrated in cell culture and in thin tissue sections, it remains difficult to use such technologies in large 3-D tissues such as brain circuits - especially if nanoscale resolution imaging is desired. Recently we developed an approach to physically magnify tissues, which we call expansion microscopy (ExM, Science 347:543-548). Expansion microscopy not only isotropically (e.g., with ~60 nm resolution) magnifies tissues, enabling super-resolution imaging on fast diffraction-limited microscopes, but also surrounds anchored biomolecules with a homogeneous aqueous environment appropriate for fast serial multiplexed tag exchanges. Here we present a suite of tools utilizing this principle, based on serial in-situ hybridization, that enable the facile, multiplexed, and super-resolved imaging of transcripts and proteins in thick (> 100 microns) brain tissue sections. We anchor both native RNA as well as antibody-targeted DNA barcodes to the ExM polymer, then perform rapid serial *in situ* hybridization using probes against these nucleic acid sequences to read out the identity and location of both native RNAs as well as DNA barcodes targeted via antibodies. Using these strategies, we demonstrate nanoscale imaging of several transcripts and proteins in brain tissue specimens. We anticipate that these methods can be used for transcriptomic and proteomic profiling of neuronal cell-types in-situ, as well as for the super-resolved characterization of neuronal connectivity and synaptic organization in intact brain circuits, key for an integrative understanding of the mechanisms underlying neural circuit function and dysfunction.

Disclosures: **F. Chen:** None. **A.T. Wassie:** None. **S. Alon:** None. **E.S. Boyden:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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NIH Director’s Pioneer Award 1DP1NS087724

NIH Director’s Transformative Research Award 1R01MH103910-01

New York Stem Cell Foundation-Robertson Investigator Award

MIT Center for Brains, Minds, and Machines

NSF CCF-1231216

Jeremy and Joyce Wertheimer

Title: Expansion microscopy in zebrafish

Authors: *L. FREIFELD[1], O. RANDLETT[2], I. ODSTRCIL[2], D. MARTIN-ALARCON[1], J. GAGNON[2], A. SCHIER[2], F. ENGERT[2], E. BOYDEN[1];
[1]Media Arts and Sci., MIT, Cambridge, MA; [2]Harvard Univ., Cambridge, MA

Abstract: We recently discovered that it was possible to physically magnify samples by embedding them in a dense swellable polymer, performing a series of chemical treatments, and then swelling the polymer (and thus the sample), a process we call expansion microscopy (ExM; Science 347(6221):543-548). Thus, one can image large 3-D samples, e.g. entire brain circuits, with nanoscale precision using ordinary diffraction-limited microscopes. Expansion microscopy enables imaging of fine details of neural projections and synaptic protein distributions, while preserving the contextual information of entire cell and circuit structures. We here report on the application of expansion microscopy to the study of the larval zebrafish, a key organism in neuroscience. The larval zebrafish is a transparent vertebrate whose brain spans only ~500μm X 500μm X 1000μm in volume. We expand entire brains of 5-7 dpf larval zebrafish and examine the detailed structure of different neural populations at multiple spatial resolutions throughout the entire brain. We follow neural projections across brain regions and describe their fine inter-region distributions and synaptic contacts at a resolution exceeding the diffraction limit, across volumes that span the entire central nervous system. As this vertebrate model lends itself to whole-brain functional imaging (Ahrens et al., 2012, Ahrens et al., 2013, Prevedel et al., 2014), applying expansion microscopy to larval zebrafish may enable rapid analysis of connectivity information. This, in the context of interpreting neural activity, may facilitate better mechanistic understanding of neural information processing in complete neural circuits. We also demonstrate expansion microscopy of zebrafish embryos, enabling high-resolution imaging of different developmental stages. (Freifeld, Randlett, Odstrcil, Martin-Alarcon and Gagnon have equally contributed to this work)

Disclosures: **L. Freifeld:** None. **O. Randlett:** None. **I. Odstrcil:** None. **D. Martin-Alarcon:** None. **J. Gagnon:** None. **A. Schier:** None. **F. Engert:** None. **E. Boyden:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSF CBET 1344219

Title: Spatial multiplexing for simultaneous imaging of multiple signaling pathways in a living cell

Authors: *G. XU, K. PIATKEVICH, K. ADAMALA, E. BOYDEN;
MIT Media Lab., Cambridge, MA

Abstract: Monitoring multiple signals at once in a living cell is challenging because the emission spectra of fluorescent biological reporters are limited to a few colors, e.g. green or red. By spatially multiplexing multiple reporters, however, we could in principle scale up the number of distinct signals simultaneously being monitored in a single cell to very large numbers, because the identity of each signaling pathway being reported upon would be encoded by the spatial position of the corresponding reporter within the cell - even if the fluorescent reporters themselves emit the same color of light. Here we design such a spatial multiplexing system, which targets fluorescent reporters that optically indicate different cell signaling pathways, to different sites in the cell. This system potentially offers the capacity for 10-20 multiplexed signals to be imaged simultaneously in mammalian cells, using reporters even of a single emission color, allowing high-content live cell imaging with commonly used epi-fluorescent microscopes. We are currently exploring targeting both ionic sensors (e.g. Ca2+, Cl- sensors) as well as kinase sensors (e.g. PKA sensors) to defined sites within neurons, with the goal of opening up the ability to survey a wide variety of signaling pathways involved with neural plasticity simultaneously within a single living neuron. We are testing the method in both primary hippocampal mouse neurons as well as human HEK293 cells, with an aim towards eventual *in vivo* use. By bringing spatial multiplexing into live cell biology, we will open up the ability to image many signals at once in living cells and organisms, providing key insights into how multiple signaling cascades work together to implement living functions.

Disclosures: **G. Xu:** None. **K. Piatkevich:** None. **K. Adamala:** None. **E. Boyden:** None.

Poster

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Support: NIH 1R01MH103910-01

Simons Center for the Social Brain

MIT Media Lab

NSF GRFP

NIH 1DP1NS087724

Title: Next-generation expansion microscopy: 20-nm resolution imaging via physical specimen magnification

Authors: *J.-B. CHANG[1], F. CHEN[1], E. JUNG[1], H. BABCOCK[2], X. ZHUANG[2], E. BOYDEN[1];
[1]MIT, Cambridge, MA; [2]Harvard, Cambridge, MA

Abstract: The identification and localization of proteins and other biomolecules, throughout entire brain circuits, with nanoscale precision would enable many fundamental insights into the mechanisms underlying the operation of normal and pathological neural networks. We recently discovered that we could physically magnify specimens by embedding them in a dense swellable polymer, anchoring key biomolecules to the polymer mesh, and adding water to swell the polymer, a process we call 'expansion microscopy' (ExM; Science 347(6221):543-548). Despite the high isotropy of the expansion process, the initial polymer recipe enabled just 4-4.5x expansion, or roughly 60-70 nm spatial resolution. Ideally it would be possible to improve the expansion chemistry so as to enable, ultimately, the imaging of membrane boundaries, as well as protein complexes. Here, we report on a next-generation ExM chemistry that can achieve ~15-20x physical magnification of mouse brain tissues, or 20-nm lateral resolution on conventional optical microscopes. As with the first version of ExM, next-generation ExM-processed samples are optically clear. Thus, next-generation ExM may be useful for imaging nanoscale neuronal structures such as synaptic clefts or synaptic vesicles over entire neural circuits in intact mammalian tissues. Brain circuit mapping using next-generation ExM may open up a variety of insights into the underpinnings of behavior, cognition, and disease. We continue to refine the chemistry and to explore how affinity tags can be adapted to work in this new expanded environment.

Disclosures: J. Chang: None. **F. Chen:** None. **E. Jung:** None. **H. Babcock:** None. **X. Zhuang:** None. **E. Boyden:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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NYSCF-Robertson Award

Synthetic Intelligence Fund

MIT Media Lab

NSF Fellowship

Hertz Foundation Fellowship

Title: Expansion Sequencing (ExSEQ): comprehensive *in situ* transcriptome characterization throughout intact brain circuits

Authors: *S. ALON[1], F. CHEN[1], E. R. DAUGHARTHY[2], P. W. TILLBERG[1], A. H. MARBLESTONE[1], A. T. WASSIE[1], G. M. CHURCH[2], E. S. BOYDEN[1];
[1]Media Lab., MIT, Cambridge, MA; [2]Wyss Inst., Harvard Med. Sch., Boston, MA

Abstract: Enabling the mapping of the cell types of the brain, as well as the systematic analysis of cell types in complex behavioral and disease states, would benefit greatly from a technology for the comprehensive analysis of gene expression patterns in neurons throughout a neural circuit. Ideally we could perform this analysis in intact brain circuits, so that these transcriptional profiles could be combined with morphological and circuit topology information, resulting in unified pictures of the cell types and cell states of the brain. Current tools do not permit this: optical methods maintain the spatial location of molecules, but the number of molecules that can be studied simultaneously is limited. On the other hand transcriptomic approaches allow the multiplexed measurement of potentially all the RNA molecules, but spatial information is lost in the process. Here we devise a new method for *in situ* sequencing of nucleic acids throughout all the neurons of an intact brain circuit, by creating new forms of expansion microscopy (ExM), a technology we previously developed that physically magnifies brain tissues while preserving nanoscale isotropy (Science 347:543-548), as well as fluorescent *in situ* sequencing (FISSEQ; Science 343:1360-1363). Using this new technology, which we call expansion sequencing (ExSEQ), users can expand brain circuits, then sequence the RNAs within the expanded tissue, resolving transcripts throughout entire neurons and neural circuits, enabling systematic cell type and cell state classification in health and disease.

Disclosures: **S. Alon:** None. **F. Chen:** None. **E.R. Daugharthy:** None. **P.W. Tillberg:** None. **A.H. Marblestone:** None. **A.T. Wassie:** None. **G.M. Church:** None. **E.S. Boyden:** None.

Poster

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NINDS 1R01S081716

NIMH 5R00MH085944

PEW FOUNDATION

ALFRED SLOAN FOUNDATION

Title: Expansion microscopy of human and nonhuman primate brain specimens

Authors: *S. S. CHA[1], A. QUACH[1], H.-A. TSENG[1], J. ZHOU[1], F. MORTAZAVI[2], K. HANSEN[1], F. CHEN[4], P. W. TILLBERG[4], R. H. MYERS[3], D. L. ROSENE[2], E. S. BOYDEN[4], X. HAN[1];
[1]Dept. of Biomed. Engin., Boston Univ., Boston, MA; [2]Dept. of Anat. & Neurobio., [3]Dept. of Neurol., Boston Univ. Sch. of Med., Boston, MA; [4]Dept. of Biol. Engineering, McGovern Institute, and Media Lab., Massachussetts Inst. of Technol., Boston, MA

Abstract: Expansion microscopy (ExM) is a recently developed technology that physically magnifies tissues isotropically by ~4-4.5x, and thus enables nanoscale super-resolution imaging of large 3-D specimens using conventional, high-speed, diffraction limited microscopes (Science 347(6221):543-548). The simple tissue preparation procedure of ExM, i.e. embedding intact tissues in networks of swellable polymers, in conjunction with conventional histological tissue handling procedures (e.g., antibody labeling) can be easily applied to various tissue types. Applied to human brain samples, ExM could potentially enable new forms of circuit-level

disease classification, diagnosis, and therapeutic target identification. Here, we use ExM to perform super-resolution imaging of non-human primate as well as human brain tissues. We demonstrate the ability to image multiple proteins in intact neural circuits from multiple brain regions, in normal and pathological brains, using conventional antibodies and fluorophores. The ability to perform super-resolution imaging of human and non-human primate brain tissues will dramatically advance our ability to understand human disease mechanisms, expand post-mortem confirmation and differential diagnosis, and enhance the identification of pathways implicated in the development of novel therapies for many brain disorders.

Disclosures: **S.S. Cha:** None. **A. Quach:** None. **H. Tseng:** None. **J. Zhou:** None. **F. Mortazavi:** None. **K. Hansen:** None. **F. Chen:** None. **P.W. Tillberg:** None. **R.H. Myers:** None. **D.L. Rosene:** None. **E.S. Boyden:** None. **X. Han:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 734.15/DD31

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH 1U01MH106011-01

NIH Director's Pioneer Award 1DP1NS087724

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New York Stem Cell Foundation-Robertson Investigator Award

MIT Center for Brains, Minds, and Machines NSF CCF-1231216

Jeremy and Joyce Wertheimer

Google

Title: Protein retention expansion microscopy

Authors: ***P. W. TILLBERG**[1], F. CHEN[2], J. YU[2], K. PIATKEVICH[3], E. BOYDEN[3]; [2]Bioengineering, [3]Media Lab., [1]MIT, Cambridge, MA

Abstract: Expansion microscopy (ExM) is a method we recently developed for physically magnifying biological samples, which enables 3-D super-resolution imaging of tissue specimens

over large volumes relevant to neural circuits (Science 347(6221):543-548). One limitation of our first implementation of expansion microscopy is that antibodies must be delivered pre-expansion, followed by gelation and strong proteolytic digestion; only the fluorophores, which are anchored directly to the gel, are physically moved apart. If, in contrast, native proteins could instead be directly anchored to the swellable gel, and the antibodies delivered post-expansion, this could enable more comprehensive staining, and more detailed interrogation of biological macromolecules in intact tissues. In addition, the resolution of the expansion microscopy process may be improved, since the size of the antibodies, which normally adds noise to the localization of the biomolecule being tagged, would effectively be divided by the expansion factor. We here report a variant of ExM in which we anchor native proteins to the expandable polymer, followed by a novel treatment strategy that enables uniform expansion. We find, using this protein retention ExM technique (proExM), that native proteins are retained in the expanded specimen, and can be stained with antibodies post-expansion. We demonstrate that, in accord with expectations, staining speed, depth of penetration, and brightness are enhanced relative to the original ExM protocol and standard immunofluorescence protocols.

Disclosures: **P.W. Tillberg:** None. **F. Chen:** None. **J. Yu:** None. **K. Piatkevich:** None. **E. Boyden:** None.

Poster

734. Whole-Brain Imaging and Atlasing II

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Jeremy and Joyce Wertheimer

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The New York Stem Cell Foundation-Robertson Award

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NIH Director's Pioneer Award 1DP1NS087724

Title: A modular protein toolbox for RNA targeting

Authors: *D. A. MARTIN ALARCON[1], K. ADAMALA[2], K. GUTHRIE-HONEA[1], E. S. BOYDEN[3];
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Abstract: The ability to monitor and perturb RNA in living neurons--which would open up the investigation of many processes that contribute to development, plasticity, and disease progression--would benefit greatly from a method of systematically targeting unmodified RNA sequences for observation and control. We report that the RNA-binding protein PumHD (Pumilio homology domain), which has been widely used in native and modified form for targeting RNA, can be engineered to yield a set of four canonical protein modules, each of which binds to one RNA base with high specificity and fidelity. We call these modules Pumby (Pumilio-based assembly) modules. Pumby modules can be concatenated in chains of varying composition and length, to bind desired target RNAs with the same behavior as PumHD but capable of binding to longer targets. We demonstrate, using Pumby chains as well as PumHD variants, RNA-directed protein assembly, quantification of RNA translation in living cells, and enhancement of translation of RNAs, without the need for appending artificial sequences to the target RNAs. These modules may prove useful for many applications in the imaging, manipulation, and biotechnological utilization of endogenous RNA targets in intact neurons and neural circuits.

Disclosures: D.A. Martin Alarcon: None. **K. Adamala:** None. **K. Guthrie-Honea:** None. **E.S. Boyden:** None.

Poster

735. Neuroanatomy: Automated Analysis

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 735.01/DD33

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH NINS/NIMH 1R01NS092474 (TRA)

Title: Synaptomes of electrophysiologically characterized human neocortical neurons

Authors: *K. D. MICHEVA[1], A. KO[2], E. LEIN[3,2], D. V. MADISON[1], A. DIJKSTRA[4], W. SEELEY[4], S. J. SMITH[3], G. TAMAS[5], J. TING[3], N. A. O'ROURKE[1];

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Abstract: The extraordinary complexity and variability of the human brain sets very high requirements for experimental methods suitable to meaningful exploration. Even when focusing on one specific neuron type within a specific brain region, variation within populations can obscure the relationship between function and underlying structure. To address such problems, we have developed a novel correlative electrophysiology-array tomography (ATomo) method to investigate the anatomical and molecular structure of individual electrophysiologically characterized neurons and their synaptic connections. Live tissue is obtained from surgical resections and prepared for patch electrophysiology. While performing whole cell intracellular recordings of individual neurons or neuronal pairs, cells are filled with Lucifer Yellow, biocytin or other fixable dyes, chemically fixed, and embedded in resin for ATomo analysis. Presence of the dye allows the subsequent localization of the physiologically characterized cells, as well as the reconstruction of their axonal and dendritic arbors. Individual input and output synapses of the filled neurons are identified and characterized by distribution, targets and molecular composition. Because ATomo enables the super-resolution imaging of dozens of different antibodies and other fluorescent markers within the same sample, filled neurons are further characterized by cytoskeletal content and presence of molecular markers such as calcium-binding proteins, while their synapses are probed for a variety of receptors and signaling molecules. Several examples will be presented, including synaptomes of inhibitory neurogliaform and chandelier cells, and excitatory pyramidal neurons. This powerful combination of electrophysiology and ATomo allows a detailed investigation of human cortical neurons by associating functional measurements with thorough descriptions of axonal and dendritic arbor morphologies and synapse distributions, sizes and molecular contents.

Disclosures: K.D. Micheva: None. **A. Ko:** None. **E. Lein:** None. **D.V. Madison:** None. **A. Dijkstra:** None. **W. Seeley:** None. **S.J. Smith:** None. **G. Tamas:** None. **J. Ting:** None. **N.A. O'Rourke:** None.

Poster

735. Neuroanatomy: Automated Analysis

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant 1R01NS092474-01

NIH Grant 1R01MH104227-01

Title: An integrated imaging and staining platform for cubic millimeter scale array tomography

Authors: *F. C. COLLMAN, S. DAVIS, O. GLIKO, T. M. KEENAN, K. PARKER, L. E. OSTROFF, S. J. SMITH;
Allen Inst. for Brain Sci., Seattle, WA

Abstract: Array tomography (ATomo) involves reconstruction of images acquired from arrays of serial ultrathin sections, which can be imaged by fluorescence microscopy (FM-ATomo) for proteometric analysis, or by scanning electron microscopy (EM-ATomo) for higher resolution. Axial resolution is defined by the thickness of physical sections (50-200 nm); the lateral resolution of FM imaging is optimal given the direct adhesion of sections to an optical coverslip. However, the throughput of data acquisition is slowed by the image acquisition time, and the need for human intervention to stain and set up samples on the microscope. The first ATomo systems acquired data at a throughput of ~18 seconds per 4 channel image (Micheva 2007). Recent work incorporated a hardware-based autofocus technology that improved throughput to 5-12 sec per 4 channel image (Rah 2013), achieving 0.1 mm^3 in 878 imaging hours with 200 nm-thick sections. Data acquisition throughput is further impaired by the overhead involved in staining and setting the sample up for imaging. As part of the Open Synaptome Project (http://opensynapto.me) effort to improve all aspects of ATomo based synaptic analysis, we are developing a next-generation system to achieve imaging throughput on the order of 0.2 mm^3 per day per microscope. We will describe the design of the imaging system, which include hardware-based autofocus, large format sCMOS sensors, motionless high intensity wide-field laser illumination, and a custom open-source software solution. We will also describe our efforts to construct a robofluidic staining solution fully integrated with the microscope. Our goal is to enable continual staining and imaging without human intervention, thus increasing both throughput and consistency of results. References: Micheva, K.D., and Smith, S.J (2007) Array tomography: A new tool for imaging the molecular architecture and ultrastructure of neural circuits. Neuron 55:25-36. Rah JC, et. Al. (2013) Thalamocortical input onto layer 5 pyramidal neurons measured using quantitative large- scale array tomography. Front Neural Circuits 7:177.

Disclosures: F.C. Collman: None. **S. Davis:** None. **O. Gliko:** None. **T.M. Keenan:** None. **K. Parker:** None. **L.E. Ostroff:** None. **S.J. Smith:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); has an founder's interest in Aratome LLC, which commercializes Array Tomography..

Poster

735. Neuroanatomy: Automated Analysis

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NIH NINS/NIMH 1R01NS092474 (TRA)

JHUAPL Internal Research and Development Grant

Title: Scalable, automated synapse detection using the open connectome project

Authors: *W. R. GRAY RONCAL[1,4], A. K. SIMHAL[5], J. T. VOGELSTEIN[2,3], F. COLLMAN[6], M. A. CHEVILLET[4], R. BURNS[1], G. SAPIRO[5], G. D. HAGER[1]; [2]Biomed. Engin., [3]Inst. for Computat. Med., [1]Johns Hopkins Univ., Baltimore, MD; [4]JHU Applied Physics Lab., Laurel, MD; [5]Duke Univ., Durham, NC; [6]Allen Inst. for Brain Sci., Seattle, WA

Abstract: New neuroimaging datasets are large (10GB-100TB), and volumes may soon exceed a petabyte for some modalities. Object detection is a canonical problem in computer vision, so a rich library of techniques is available to aid in neuroscience inference tasks. However, Big Vision requires a paradigm shift to overcome challenges such as data storage, computation and multiscale semantic understanding. We have developed open-source tools for scalable object detection (http://w.ocp.me/tools:object_detect), leveraging the Open Connectome Project (OCP, http://openconnecto.me) infrastructure and the Laboratory of Neuroimaging Pipeline processing framework. This allows users to quickly adapt their algorithms in a flexible, reproducible environment. As a case study, we demonstrate our pipeline by generating and deploying a lightweight, scalable synapse detector to find approximately 50,000 putative synapses in 60,000 μm^3 of electron microscopy data. This framework is extensible to other challenges and modalities. For example, Immunofluorencence Array Tomography (ATomo) can provide high-dimensional proteometric characterization of large populations of synapses, supplementing the information available with EM imaging. ATomo is a high-throughput approach, requiring scalable infrastructure. To ensure maintainability, reproducibility, and comprehensive testing across datasets and techniques, we used the OCP framework to create a common infrastructure to support ATomo based synapse detection. Manual and computer detected synapses are uploaded to OCP and interactively viewable via 2D Web-services. We are scaling up the synapse detection work for IF synapses, leveraging the framework used to detect synapses in EM data as part of the Open Synaptome Project (http://opensynapto.me).

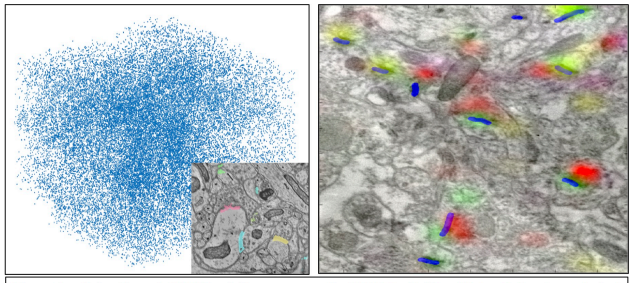

Figure 1: Detection of 50,000 putative synapses in EM Data (left), with inset showing selected individual detections. Array Tomography IF Data (right); shows an EM slice (in grayscale) with IF channels overlaid: PSD-95 (green), synapsin (red), VGluT1 (pink), NR1 (yellow). The EM identified synaptic clefts (manual ground truth) are highlighted in blue.

Disclosures: W.R. Gray Roncal: None. **A.K. Simhal:** None. **J.T. Vogelstein:** None. **F. Collman:** None. **M.A. Chevillet:** None. **R. Burns:** None. **G. Sapiro:** None. **G.D. Hager:** None.

Poster

735. Neuroanatomy: Automated Analysis

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: R01MH103910

NIH/NIBIB 1R01EB016411

NIH NINS/NIMH 1R01NS092474

Title: Quantifying mesoscale neuroanatomy with X-ray microtomography

Authors: *E. L. DYER[1,2], H. L. FERNANDES[1,2], X. XIAO[3], W. GRAY RONCAL[4], J. T. VOGELSTEIN[5], C. JACOBSEN[6,3], K. P. KORDING[1,2], N. KASTHURI[7];
[1]Dept. of Physical Med. and Rehabil., Rehabil. Inst. of Chicago, Chicago, IL; [2]Northwestern Univ. Med. Sch., Chicago, IL; [3]Argonne Natl. Lab., Lemont, IL; [4]Applied Physics Lab. of Johns Hopkins Univ., Baltimore, MD; [5]Biomed. Engin., The Johns Hopkins Univ., Baltimore, MD; [6]Physics and Astronomy, Northwestern Univ., Evanston, IL; [7]Boston Univ., Boston, MA

Abstract: The structure of the brain is constantly being modified due to experience, learning, aging, and in some cases, disease. Understanding the impact of such modifications on brain architecture, at various spatial scales, will have wide reaching impacts. Unfortunately, existing methods for quantifying the neuroanatomical structure of the brain, such as light and electron microscopy (EM), cannot be readily applied to large brain volumes. For this reason, there exists

an information gap at the mesoscale: we require new techniques to produce brain maps that characterize the cell shapes, densities, and positions and their long-range projections across large spatial extents. Synchotron-based X-ray microtomography (XRM) is uniquely poised to fill this gap. Compared to electrons or visible light photons, X-rays undergo very little multiple scattering so that thick objects can be studied at sub-micrometer resolution. Unfortunately, XRM has not been completely adapted to meet the demands of large-scale brain imaging. Here, we provide a host of new methods and an end-to-end pipeline for quantifying neuroanatomy from large brain volumes with XRM. Our pipeline consists of methods for: (i) sample preparation, (ii) image reconstruction from raw XRM measurements, and (iii) automated segmentation, cell detection, and spatial statistics. We integrated our data and analysis tools into the Open Connectome Project infrastructure (OCP, http://www.openconnecto.me), an open-access framework for large-scale analysis of neuroimaging datasets. We show that XRM of samples prepared for electron microscopy (i.e. aldehyde fixed, osmicated, and embedded in plastic) produces images stack with sufficient resolution (~1 micron isotropic) to identify all cell bodies, their sizes, locations and in some cases partial reconstructions of their dendritic processes. The trajectory of all the vasculature and many of the myelinated axons can also be reconstructed. Our results suggest that XRM promises a new avenue for neuroscientists to study the mesoscale architecture of large brain volumes.

Disclosures: **E.L. Dyer:** None. **H.L. Fernandes:** None. **X. Xiao:** None. **W. Gray Roncal:** None. **J.T. Vogelstein:** None. **C. Jacobsen:** None. **K.P. Kording:** None. **N. Kasthuri:** None.

Poster

735. Neuroanatomy: Automated Analysis

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant 4 R00 LM011390-02

Title: Automated GPU-accelerated segmentation of volumetric fiber networks

Authors: *P. A. GOVYADINOV, D. MAYERICH;
Electrical and Computer Engin., Univ. of Houston, Houston, TX

Abstract: High-throughput microscopy techniques such as Knife-Edge Scanning Microscopy, allow three-dimensional imaging of whole organ tissue samples at sub-cellular spatial resolution. Filaments are common structures in biomedical tissue samples, however they are extremely

difficult to image and reconstruct using standard techniques. With the gaining popularity of new techniques that can image entire tissue samples at cellular levels, segmentation methods focused on large data sets are becoming extremely important. The most common examples filament networks in tissue samples are neuronal networks and microvasculature. These examples are composed of cellular structures that form complex interconnected networks of thin fibers. Since these structures are often less than a few micrometers in diameter, high-resolution imaging is required, despite the fact that the networks embedded can comprise < 6% of the entire volume. However, existing segmentation algorithms are ill-equipped to deal with data sets that often exceed several terabytes. We present a fast, hardware accelerated predictor corrector algorithm for segmenting networks embedded in large volumetric datasets. Segmentation is performed with a filament tracking algorithm using a predictor-corrector approach. Beginning from an initial seed point, the current fiber trajectory is estimated using template matching. In order to provide fast segmentation, we take advantage of GPU texture memory for spatially cached sampling and limit out analysis to data close to the embedded network, representing a small fraction (2-6%) of the total data set. By using graphic acceleration hardware we eliminate the need for supercomputing, while increasing usability for biomedical professionals.

Disclosures: **P.A. Govyadinov:** None. **D. Mayerich:** None.

Poster

735. Neuroanatomy: Automated Analysis

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant 4 R00 LM011390-02

Title: Fast automated segmentation of neural soma in large KESM images of brain tissue

Authors: ***L. SAADATIFARD**[1], Y. CHOE[2], L. ABBOTT[2], D. MAYERICH[1];
[1]Univ. of Houston, Houston, TX; [2]Texas A&M Univ., College Station, TX

Abstract: Knife-Edge Scanning Microscopy (KESM) allows researchers to quickly collect terabytes of three-dimensional tissue data. This provides the potential for extracting detailed information about cell positions and connectivity across large (cubic centimeter) volumes of tissue. However, the size and complexity of these volumetric data sets makes segmentation difficult. The size of the acquired KESM data makes any form of manual intervention in the segmentation impractical. Therefore, fully automated techniques are required in order to

realistically process these data sets. Maintaining high accuracy with minimal user input is a well understood problem in segmentation. In this presentation, we will describe a fully automated method for finding the position of cell soma in KESM images stained en bloc using thionin. An iterative voting technique is used to generate a binary segmentation of the volumetric data, which is then refined to determine the location of cell soma. This technique requires minimal user input and no user feedback, making it amenable to fully automated processing of terabyte-scale data sets. We demonstrate the effectiveness of this algorithm on volumetric images of the rat somatosensory cortex obtained using KESM by comparing to a manually segmented ground truth. In addition, the proposed algorithm can be readily parallelized in order to provide efficient evaluation using heterogeneous (GPU-based) hardware commonly available in commercial computer systems.

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Poster

735. Neuroanatomy: Automated Analysis

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant MH093011

Pritzker Neuropsychiatric Research Consortium

NIH Grant MH099721

Title: Automatic reconstruction of neurons and vessels in CLARITY-cleared specimens

Authors: ***S. TAPPAN**[1], D. M. KROLEWSKI[2], B. MARTIN[2], M. A. A. KARIM[1], D. HOPPES[1], N. ROUSSEL[1], P. J. ANGSTMAN[1], S. J. WATSON, Jr.[2], J. R. GLASER[1]; [1]R&D, MBF Biosci. - MicroBrightField Inc., Williston, VT; [2]Mol. and Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: CLARITY and other related tissue clearing agents have opened the possibility for unprecedented studies of neuroanatomy and cytoarchitecture by removing the necessity of tissue sectioning. As imaging methodologies evolve for these intact specimens it is also evident that specialized quantitative tools are needed. CLARITY-cleared specimens are often multi-millimeter thick, which require sophisticated and expensive microscopy equipment (e.g., COLM microscopy and super long working distance objective lenses) and long duration acquisition

times. The resulting image data can be very large (>0.5 TB). Further, the focus resolution limitations due to the objectives generally result in voxel dimensions that are challenging for automated reconstruction. As such, quantitative software that can analyze CLARITY image data faces special challenges. To balance the exceptionally long imaging times, quantitative data extraction must be fast and robust. In the current study, we demonstrate the utility of CLARITY image data and the functionality of specialized quantitative software. Decades of research indicate a critical role of the microvasculature in the plasticity of the brain under various physiological and pathological conditions, including fundamental processes during brain development, learning, recovery from traumatic brain injury and brain inflammation, aging and neurodegeneration. Tissue cleared with CLARITY offers the opportunity to investigate these processes in detail. We used Neurolucida 360 to visualize and quantify neuronal structures in large image volumes. In addition, preliminary data from new prototype software, Vesselucida, for reconstruction of microvasculature is presented. These novel automated methods are computationally efficient and therefore greatly reduce the time needed for analysis. Image management is a central concern, because there is a simultaneous need to have the ability to interact with the entire image volume and a small subset that represents the current region of interest. Neurolucida 360 and Vesselucida address this need by using an interactive 3D visualization environment that enables multi-resolution image handling. Real-time volume rendering of large amounts of data using non-specialized computer hardware is then used to elucidate spatial orientation. Automated methods for reconstructing neuronal features across multiple fields of view further streamline the data acquisition. This new development now makes it possible to perform automated quantitative analyses at the maximal resolution of the image volume while having the morphological and organizational context of the intact tissue specimen.

Disclosures: **S. Tappan:** A. Employment/Salary (full or part-time):; MBF Bioscience (full-time). **D.M. Krolewski:** None. **B. Martin:** None. **M.A.A. Karim:** A. Employment/Salary (full or part-time):; MBF Bioscience (full-time). **D. Hoppes:** A. Employment/Salary (full or part-time):; MBF Bioscience (full-time). **N. Roussel:** A. Employment/Salary (full or part-time):; MBF Bioscience (full-time). **P.J. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience (full-time). **S.J. Watson:** None. **J.R. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience (full-time). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MBF Bioscience - MicroBrightField, Inc..

Poster

735. Neuroanatomy: Automated Analysis

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: 9R44MH076541

Florida High Tech Corridor Grant

Byrd Research Center Grant

Title: Automatic stereology of substantia nigra using a novel segmentation framework based on the balloon active contour model

Authors: *P. R. MOUTON[1], P. A. PHOULADY[2], L. O. HALL[2], D. GOLDGOF[2];
[1]Pathology & Cell Biol., Univ. of South Florida Col. of Med., Tampa, FL; [2]Engin. & Computer Sci., Univ. of South Florida, Tampa, FL

Abstract: The use of manual (non-automated) computer-assisted approaches to collect stereology data from stained neural structures can be tedious, time-consuming and subject to bias from user fatigue. Feature-based segmentation algorithms holds great promise for quantifying stereology parameters with higher throughput efficiency without a loss of accuracy. The present study proposes a novel segmentation framework using an Active Contour Model (balloon snakes) to automate area and volume quantification by the point-counting method. Balloon snakes are energy minimizing splines that conform to the boundaries of arbitrary shaped objects. Energy is derived from external forces driven from the image and internal forces such as elasticity and stiffness of the snake. This approach is demonstrated for three month-old Fisher 344 rats with experimental Parkinsonism (n = 2) and vehicle control (n = 4). Brains were perfused in-vivo with aldehydes and frozen sections cut at 30 um thickness. From each rat brain 12 sections were sampled in a systematic-random manner through the entire substantia nigra pars compacta (SN) and immunostained for tyrosine hydroxylase (TH)-positive neurons. Ground truth data from manual stereology was collected using a computerized stereology system (Stereologer, SRC, Tampa, FL). For the automatic framework, the system's motorized xyz stage automatically collected between 500 and 1000 images through the entire SN at high magnification (100x oil). The novel framework was applied to segment TH-positive neuronal cell bodies using a balloon active contour model with non-constant balloon force. After 200 iterations, several contours were initialized within the images and classified as TH-positive cell bodies (signal) or background. Cell contours were automatically selected based on predefined inclusion/exclusion criteria (e.g., area of contour, dispersion measures, degree of overlap) and the total area and volume of TH-positive neurons automatically calculated by the system software. There was a strong correlation (R2 >/=0.95) for total area and volume of TH-positive neurons by the automatic framework as compared with ground truth by manual stereology, with a ten-fold increase in efficiency as quantified by reduced time for the automatic framework to achieve the same level of precision as manual stereology. In light of the enhanced efficiency and

comparable accuracy with the automatic balloon active contour framework, these findings strongly support continued development of pattern recognition algorithms for automatic stereology of neural structures.

Disclosures: **P.R. Mouton:** A. Employment/Salary (full or part-time):; Stereology Resource Center. **P.A. Phoulady:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of South Florida. **L.O. Hall:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of South Florida. **D. Goldgof:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of South Florida.

Poster

735. Neuroanatomy: Automated Analysis

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Program#/Poster#: 735.10/DD41

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: nTracer: An ImageJ software package for neural circuit reconstruction and analysis from multi-spectral 3D images

Authors: *D. H. ROOSSIEN, JR[1], A. S. DIZAJI[1], R. HUTH[1], J. STECHER[1], J. WEBB[2], L. BOGART[3], T. HENSCH[3], E. D. HERZOG[2], D. CAI[1];
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Abstract: The biological principle that structure determines function is perhaps most evident in the brain. As such, the ability to reconstruct neuronal networks is a central goal in modern neuroscience, be it of local circuits or an entire connectome. A notable challenge is to extract intuitive and quantifiable information from the wealth of images that record the microscopic structures of neural circuits. Several software platforms have been developed that enable the reconstruction of cell morphologies in “Cartesian” descriptions. However, these have been limited to single cells in sparsely labeled samples to avoid ambiguities between neurites in close proximity, thereby prohibiting analysis of connectivity. Recent advances in multicolor genetic labeling strategies, such as Brainbow, offer advantages over traditional single color/tracer approaches by being able to identify multiple cells in a sample based on unique colors with

limited redundancy. Hence we developed nTracer, a user-guided ImageJ plugin with the ability to trace multiple neurons in a densely labeled brain tissue based on color profile. Being able to mark synaptic locations and establishing connections between neurons, nTracer allows the user to analyze circuit connectivity. The software includes a user-friendly output interface, which can reconstruct a customizable 3D rendering of a traced circuit based on morphology, cell type, connectivity, or any other custom parameter(s) and can export tracing results of single neurons in SWC format for use with currently available morphometric tools. To demonstrate the software's versatility, we traced a variety of densely-labeled neurons throughout the brain. Specifically, we traced granule cells in the dentate gyrus and hypothalamus, vasoactive intestinal peptide (VIP)-expressing inhibitory neurons in the suprachiasmatic nucleus, basket cells and pyramidal cells in the CA1 region of the hippocampus, or cholinergic excitatory neurons in the striatum. The unambiguously traced profiles allowed us to perform morphology-based neuronal subtype classification. Furthermore, we were able to provide direct measurement for how basket cells assemble into CA1 circuits in terms of total number of cells making synaptic connections onto excitatory pyramidal cells and other basket cells. nTracer is thus the first software package with the ability to answer questions requiring reconstruction and analysis of single neurons to complex circuits. Moreover, nTracer results provide a ground truth for validating automated 3D neuronal tracing algorithms applicable to densely labeled tissue, the development of which remains a major long-term goal for the field.

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Poster

735. Neuroanatomy: Automated Analysis

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Topic: G.03. Staining, Tracing, and Imaging Techniques

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ONR MURI 14101-0198

Keck NAKFI

Title: NeuroMorpho.Org: connecting neuronal morphology with digital neuroscience

Authors: *R. ARMANANZAS, R. PAREKH, S. POLAVARAM, S. NANDA, G. A. ASCOLI; Krasnow Inst. For Advanced Study, George Mason Univ., Fairfax, VA

Abstract: Distributed multidisciplinary collaborations produced many success stories in 21st century science. Leveraging the Internet model of hyperlinked information, molecular biology rapidly expanded in the early 2000s from isolated wet labs to online repositories of shared digital data. Neuroscience is now similarly evolving into a networked ecosystem, integrating and augmenting individual research projects with big data initiatives. NeuroMorpho.Org is to date the largest freely accessible online repository of digital reconstructions of neuronal morphology. Version 6.1 (May 2015) included 31,983 reconstructions from 24 species and 139 brain regions contributed by 147 laboratories worldwide. Axonal and dendritic reconstructions are useful for neuronal identification, compartmental models, and circuit analysis, but accurate metadata are necessary for proper interpretation. NeuroMorpho.Org cross-links digital reconstructions with BrainInfo.org, providing valuable details about mammalian brain regions. An effective computational modeling pipeline is achieved by links to NeuroMorpho.org entries from the SenseLab repositories ModelDB and NeuronDB. Continuous collaboration with the BigNeuron initiative for automated tracing is coalescing into a virtual neuromorphological data network. Interlinking data with the relevant source original articles is also essential. NeuroMorpho.Org maintains a literature database of ~25,000 neuroscience publications identified by full-text searches, indexed by PubMed IDs, and mined for digital reconstruction content. When digital morphologies are uploaded to the repository, each PubMed entry is linked (via Neuroscience Information Framework) to its reconstructions and vice versa. Publishers can promote data sharing and facilitate access to available data. In an ongoing process, NeuroMorpho.Org and Elsevier are reciprocally cross-linking articles and corresponding reconstructions by Digital Object Identifier (DOI). Cross-integration of NeuroMorpho.Org with an increasing number of complementary resources creates a knowledge network providing not only data content, but also rich contextual information.

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Poster

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Support: the Funding Program for Next Generation World-Leading Researchers, Grant No. LS081

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Research Fellowships for Young Scientists

Title: A new block-face serial microscopy tomography for computational mapping of brain cells, and an unbiased comparative analysis

Authors: *K. SEIRIKI[1], A. KASAI[2], T. HASHIMOTO[3], W. SCHULZE[2], M. NIU[2], T. NAKAZAWA[2], H. HASHIMOTO[2];
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Abstract: Identification of anatomical alterations and functional activation patterns in a systemic manner is of the greatest importance to understand brain function and dysfunction. To this end, several whole brain imaging techniques such as light-sheet fluorescent microscopy with tissue-clearing methods has emerged last few years. Despite of these great advances in microscopic techniques, it is still challenging to properly detect these alterations in even the whole brain of mouse by a quantitative comparison. Here, we developed a high-speed imaging apparatus based on automated mechanical sectioning and confocal imaging, named FAST (blockface serial microscopy tomography), and image data processing pipeline for 3D image construction without denaturing (e.g. tissue-clearing techniques). Image processing in FAST could identify individual fluorescent signals and analyze the distribution in the whole brain. We imaged whole mouse brains stained with hoechst33258 using FAST and acquired spatial information of cell distribution. To achieve detection of the structural alterations from whole brain data, we established semi-automatic registration with FAST processing system, and detected the loss of cells in a neurodegeneration model which shows particularly in injured dentate gyrus. Moreover, FAST is also applicable to conventional histological studies using stored sections after whole brain imaging, because this system does not need any denaturing of tissues for whole brain imaging. Including conventional histological methods, various subsequent applications in FAST could provide more useful information, such as cell type in the altered brain regions. Therefore, FAST could identify anatomical and functional alterations, much of which have been overlooked in conventional hypothetical studies, and unbiased comparison with FAST will contributes to further understanding of brain function and dysfunction.

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Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Methods for reconstructing 3D brain data from histological sections for making axonal projection maps in the marmoset

Authors: *H. ABE[1], T. TANI[1], H. MASHIKO[1], N. MIYAKAWA[2], K. MIMURA[2], K. SAKAI[2], W. SUZUKI[2], T. KUROTANI[1], N. ICHINOHE[2,1];
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Abstract: The common marmoset (Callithrix jacchus) is becoming a popular non-human primate model in neuroscience research due to several advantages including the well-developed prefrontal cortex and the availability of transgenic technologies. Because its brain connectivity is poorly understood, it is necessary to collect and present data in comprehensible ways as a marmoset brain connectivity atlas to further enhance neuroscience research. The previous anatomical studies using tracers often show results only from a part of the brain which may not meet readers' own interests. And a brain atlas needs a 3D coordinate, but histologically processed sections have no obvious coordinate across the sections on the glass-slides due to mounting and the tissue shrinkage. To overcome these problems, the present study is aimed to make axonal projection maps of the whole marmoset brain using a non-rigid registration method implemented in Advanced Normalization Tools. Axons were labeled by virus tracers encoding fluorescent proteins. Bain sections were examined with a fluorescent microscope for the axonal projections and with myelin and Nissl staining for brain annotations, and were integrated into a common 3D space for each animal. To achieve this, every time before a section was sliced with a sliding freezing microtome, a picture of the coronal section of the remaining brain was taken using a camera fixed above, to have a 2D coordinate held across the pictures. Additionally the slice thickness gives information about the orthogonal axis, which results in having a common 3D space. The brain images segmented from the pictures were used as reference images to register the corresponding brain sections into the 3D space using the registration method. Thus axonal projections and brain annotations based on the staining can be integrated. In addition, for easier brain segmentation, a picric acid was added to a PFA solution to make the brain yellowish since otherwise the frozen brain looks similar in the color to the surrounding dry ice. To enhance fluorescent signals, images were taken with an additional dummy channel and processed with independent component analysis. This signal processing technique effectively separated signals from the fluorescent proteins and from the background auto-fluorescence which appeared similarly across the channels. We applied these methods to marmoset brains and successfully reconstructed axonal projections from multiple areas in the frontal, temporal and occipital cortices.

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Poster

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Title: Automating the analysis of large-scale electron microscopy image stacks using scalable workflows and high performance computing

Authors: ***A. J. PEREZ**[1,2,3,4], C. CHURAS[1,2,3,4], W. WONG[1,2,3], M. CHIU[1,2], K.-Y. KIM[1,2,3], E. A. BUSHONG[1,2,3], T. J. DEERINCK[1,2,3], S. PANDA[6], T. TASDIZEN[7], M. H. ELLISMAN[1,2,3,4,5];
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Abstract: The quantification of biologically relevant data from 3D electron microscopy (EM) datasets is a long-standing bottleneck for the study of nervous system form and function. Such analyses typically rely upon manual segmentation, a labor-intensive process that does not scale to accommodate the rapidly increasing throughputs afforded by techniques such as serial block-face scanning EM or electron tomography. Though much effort has been spent developing methods to automate segmentation processes, many solutions remain inaccessible to the research community due to steep learning curves or difficulty in gaining access to high performance computing resources (HPC). A web portal was developed that employs semi-automatic segmentation solutions into scalable Kepler workflows for HPC, addressing this issue. This approach reduces complexity for the user by wrapping complicated, multi-step computational processes into single pipelines that can be submitted by a simple mouse click. Once initiated,

segmentation jobs are processed in parallel and distributed across numerous geographically distributed HPC resources using a multi-cluster submission system. Following this processing, workflows for object filtering, contour generation, and tetrahedral mesh generation are available. Our current segmentation system utilizes the cascaded hierarchical model (CHM), a supervised machine learning algorithm, to produce voxel probability maps of input data. These probability maps are then segmented by the evolution of automatically seeded active contours, and 3D reconstructions are computed using the IMOD software package. Organelle-specific quantification routines have been developed to automatically compute numerous, advanced morphological descriptors, including mitochondrial branching and branch length and nuclear envelope curvature. Such routines will be incorporated into the web portal, providing the user with an end-to-end process beginning with raw images and ending with quantitative data and cellular models. These methods simplify and expedite the process of developing and quantifying complete microanatomical models of cells, and can be used, for example, to study the effects of neurodegenerative diseases on organelle morphology and distribution. The web portal and related services are available at https://slashsegmentation.com.

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Poster

735. Neuroanatomy: Automated Analysis

Location: Hall A

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Program#/Poster#: 735.15/DD46

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Deconvolution in sem - enhancing resolution in x, y and z

Authors: ***B. H. LICH**[1], F. BOUGHORBEL[1], P. POTOCEK[1], E. KORKMAZ[1], M. LANGHORST[2];
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Abstract: In recent years there has been a considerable advancement in SEM-based methods for 3D reconstruction of large tissue volumes. Serial Block-Face SEM (SBF-SEM) involves combination of imaging and in-situ sectioning of plastic embedded tissue blocks within the SEM vacuum chamber1, allowing for automated imaging and subsequent reconstruction of volumes of tissue. The use of low electron energies for imaging limits sample charging which can be further mitigated with imaging in low vacuum mode and by further increasing the conductivity of the

sample through sufficient amount of heavy metal staining3. Last year we introduced a novel solution for high spatial resolution and throughput SEM volume imaging overcoming the resolution limits set by mechanical slicing by combining it with virtual sectioning. Virtual slicing is realized by Multi-Energy Deconvolution SEM (MED-SEM), a non-destructive technique that allows high resolution reconstruction of the top layers of the sample.2 After cutting a thin layer of the blockface using a diamond knife, freshly exposed tissue is imaged several times using various accelerating voltages. These images are subsequently used for deconvolving the information into several virtual subsurface layers. This cycle of physical and virtual sectioning offers isotropic datasets with excellent z-resolution and are fully integrated and automated. Here we introduce novelties on how different image acquisition and processing (deconvolution) methods help to achieve goals that are also very relevant for neuroscientists. For instance achieving enhanced resolution, not only in the orthogonal plan like we have demonstrated with multi-energy deconvolution, but also in the x-y (image) plane. We can achieve this in various ways but here we demonstrate how this can conceptually be achieved by dedicated detector design and by combining different beam-tilts.

Disclosures: **B.H. Lich:** A. Employment/Salary (full or part-time):; FEI Electron Optics BV. **F. Boughorbel:** A. Employment/Salary (full or part-time):; FEI Electron Optics BV. **P. Potocek:** A. Employment/Salary (full or part-time):; FEI Electron Optics BV. **E. Korkmaz:** A. Employment/Salary (full or part-time):; FEI Electron Optics BV. **M. Langhorst:** A. Employment/Salary (full or part-time):; FEI Munich.

Poster

735. Neuroanatomy: Automated Analysis

Location: Hall A

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Development of a correlation technique between synchrotron x-ray microtomography and transmission electron microscopy for the study of nervous system diseases

Authors: *P. PARLANTI[1,2], V. CAPPELLO[1], G. TROMBA[3], F. BRUN[3,4], I. TONAZZINI[5], M. CECCHINI[5], V. PIAZZA[1], M. GEMMI[1];
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Abstract: We report a new correlative-microscopy technique that combines two highly complementary imaging techniques, synchrotron X-ray microtomography (microCT) and transmission electron microscopy (TEM), giving unprecedented access to the tridimensional reconstruction of a histological sample with details down to the microscopic level as well as to its nanometer-scale ultrastructure. To this end we identified fixative and staining procedures that rendered the sample of interest observable with both these techniques by preserving the ultrastructure for TEM analysis while yielding at the same time optimal contrast for microCT imaging. We demonstrate the potency of this technique by targeting pathological changes arising in the sciatic nerves of Twitcher mice (TWI), a murine model of globoid cell leukodistrophy (GLD), also known as Krabbe disease. GLD is a genetic disease caused by the deficiency of galactocerebroside-beta-galactosidase (GALC) activity that induces the accumulation of lipid-raft associated neurotoxin psychosine and the infiltration of multinucleate (globoid) cells. We were able to identify individual infiltrating cells, a hallmark of the pathology, in the 3D rendering of the sample determining their precise position. With this knowledge, we could section the sample only in the region of interest, avoiding the time-consuming process required by conventional serial-sectioning methods, for further observation at TEM, allowing the characterization of the ultrastructure of the chosen globoid cell. The correlation technique allows to locate, with the micrometric resolution of the microCT, in a large volume all regions interested by the pathological process, and select only these for further ultrastructural characterization. The method represents an invaluable tool for the investigation of those pathologies in which the microscopic alterations are localized in few confined regions, rather than diffuse in entire tissues, organs or systems. For that reason, we are going to show that this method is applicable to samples with a larger volume, arising from different animal models.

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Poster

736. Optogenetic Studies of Neural Circuits

Location: Hall A

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Topic: G.04. Physiological Methods

Support: National Institutes of Health

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Title: Top-down bidirectional control of innate anxiety and learned fear

Authors: *A. ADHIKARI[1,2], T. N. LERNER[2], J. FINKELSTEIN[2], S.-Y. KIM[2], J. H. JENNINGS[2], L. YE[2], L. A. GUNAYDIN[2], J. MIRZABEKOV[2], S. PAK[2], A. LEI[2], K. DEISSEROTH[2,3,4];
[1]Neurobio., Stanford Univ., Palo Alto, CA; [2]Bioengineering, [3]Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; [4]Howard Hughes Med. Inst., Stanford, CA

Abstract: Previous studies showed electrical stimulation of the ventral medial prefrontal cortex (vmPFC) during cued fear extinction increases extinction retrieval. As fear conditioned responses are dependent on the central amygdala, it has been hypothesized that the vmPFC decreases fear by activating inhibitory intercalated cells in the amygdala, which in turn inhibit the central amygdala. However, this model has not been directly tested. Here, we tested these ideas directly by optogenetically activating vmPFC cell bodies and their projections to the amygdala during innate anxiety and cued and contextual fear extinction. Animals were injected with viral vectors encoding ChR2-EYFP, eNpHR3.0-EYFP or EYFP under the CamK2α promoter in the vmPFC and fiberoptic cannulae were implanted either in the vmPFC or above the amygdala. While activation of vmPFC cell bodies did not alter anxiety, optical activation of the vmPFC-amygdala projection decreased anxiety in the elevated plus maze and decreased respiration rate, which is a physiological marker of anxiety ($n=10$ EYFP, 11 ChR2, $p<0.05$). Conversely, inhibiting this projection in mice expressing eNpHR3.0 had the opposite effects ($n=8$ EYFP and 11 eNpHR3.0, $p<0.01$). Furthermore, optogenetic activation of vmPFC cell bodies ($n=7$ EYFP and 7 ChR2, $p<0.05$) during fear extinction decreased freezing specifically during cued fear extinction retrieval. Remarkably, activation of the vmPFC-amygdala projection only during fear extinction decreased freezing both during extinction and retrieval of extinction, for both cued and contextual conditioning ($n=7$ EYFP, 10 ChR2, $p<0.05$). Surprisingly, retrograde viral tracing, histology and *in vivo* and *in vitro* functional connectivity showed that the intercalated cells do not receive vmPFC projections. Instead, the basomedial amygdala was found to be the major post-synaptic target of the vmPFC in the amygdala. Lastly, direct optical activation of basomedial amygdala cell bodies decreased expression of both innate anxiety and learned cued fear conditioning ($n=8$ EYFP and 9 ChR2, $p<0.05$). Taken together, these data suggest the vmPFC-amygdala projection robustly inhibits learned fear and innate anxiety by activating the basomedial amygdala.

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Poster

736. Optogenetic Studies of Neural Circuits

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Beckman Institute for Optogenetics and CLARITY

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Caltech-GIST

Title: Anatomical and functional characterization of the rat cholinergic pedunculopontine neurons and projections to dopaminergic cells in the ventral tegmental area

Authors: *J. CHO[1], C. XIAO[2], J. TREWEEK[2], K. CHAN[2], V. GRADINARU[2];
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Abstract: The pedunculopontine nucleus (PPN) in the rostral brainstem is postulated as an important region mediating reward learning by sending excitatory signals to ventral midbrain dopaminergic neurons. The PPN consists of an intermingled population of cholinergic, glutamatergic, and GABAergic neurons. To investigate the causal role of specific PPN cell types in promoting reward seeking behavior, we used optogenetics in a transgenic rat line that expresses Cre-recombinase under choline acetyltransferase (ChAT) promoter. Cre-dependent viral tracing (AAV5-Ef1a-DIO-ChR2(H134R)-eYFP) demonstrated highly specific expression (~95%) in cholinergic neurons. Thick brain slice imaging with the passive CLARITY technique (PACT) showed that cholinergic PPN fibers innervate dopaminergic neurons in both of the ventral tegmental area (VTA) and substantia nigra pars compacta. Optogenetic activation and inhibition of cholinergic PPN cell bodies induced conditioned place preference and aversion, respectively. (PPN-ChR2, PPN-mCherry, PPN-Arch3, n=6 rats each). *In vivo* electrophysiological recordings during anesthesia demonstrated that cholinergic PPN neurons can be identified by optogenetics-based phototagging (2/13 neurons, n=2 rats), and activation of these fibers leads to increased firing rate of VTA neurons (~3 to 7 fold, 7/11 neurons, n=2 rats). These results can aid future studies aimed at investigating the causal role of cholinergic PPN inputs to VTA in reward seeking behavior.

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Poster

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Topic: G.04. Physiological Methods

Support: HHMI International Student Research Fellowship

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Gatsby Foundation

SFARI Program

Title: Optogenetic rescue of impaired social behavior phenotype in autism

Authors: ***A. SELIMBEYOGLU**[1,2], C. K. KIM[1], M. WRIGHT[3], A. S. O. HONG[4], C. RAMAKRISHNAN[4], L. E. FENNO[1], T. J. DAVIDSON[4], K. DEISSEROTH[2,3,4]; [1]Neurosciences, [2]Hhmi, [3]Psychiatry and Behavioral Sci., [4]Bioengineering, Stanford Univ., Stanford, CA

Abstract: Altered cellular excitatory/inhibitory (E/I) balance is emerging as a common pathophysiological characteristic that could link diverse autism-associated genetic variations in mouse models. To understand possible disease relevance, it may be of great interest to identify specific cells and circuits within which such E/I imbalance changes could cause autism-related phenotypes in real time. Increased excitability in medial prefrontal cortex (mPFC) pyramidal (PYR) neurons was previously found to reduce social interaction in mice, which can be partially recovered by simultaneous compensatory excitation of inhibitory parvalbumin (PV) neurons. Here, we investigate the role of real-time changes in E/I balance in a clinically-inspired CNTNAP2 KO mouse line with decreased cortical PV neurons and impaired social behavior. We crossed CNTNAP2 mice with PV::Cre mice, and expressed bistable SSFO channelrhodopsin in PV cells. Without stimulation, CNTNAP2 KO mice spent less time engaging socially than WTs ($n=7$ WT, $n=11$ KO, $p<0.05$). Increasing PV cell excitability reversed the impaired social behavior ($n=11$ KO no-stim, $n=6$ KO stim, $p<0.05$) in KOs while not affecting WT littermates

(n=9 WT no-stim, n=7 WT stim, $p>0.05$). We did not find any differences in novel object interaction between WTs and KOs, and the SSFO stimulation did not affect object interaction (n=4 WT, n=6 KO, $p>0.05$, for both stim and no-stim). The phenotype could be largely traced to decreased mean interaction time in the initial KO social bouts (n=6 KO, n=5 WT, $p<0.01$); additionally, WT social bout length decayed exponentially during the course of the test, while KO's did not (n=5 WT $R^2=0.88$, n=6 KO $R^2=0.10$). We further dissected real-time activity in mPFC by using fiber photometry to simultaneously record from PV and PYR neurons expressing ef1a-DIO-GCaMP6f and CKIIa-RCaMP2, respectively. Both types of neurons responded to novel social and object stimuli in a time-locked manner, with similar peak fluorescence changes (peak dF/F for social vs object; n=5 WT $p>0.05$, n=6 KO $p>0.05$). On the other hand, within-bout fluorescence changes were different for WT and KO mice during the initial bouts. Peak fluorescence signals were significantly different at the end of the bout compared to the beginning for WT social but not object interactions; in contrast these signals were similar for KOs (first vs last 0.25 s; n=5 WT $p<0.01$ for social, $p>0.01$ for object; n=6 KO $p>0.01$ for both social and object), suggesting that the KOs process social stimulus similarly to objects. Together these results illustrate the role of real-time balance between mPFC PV and PYR cells in modulating social behavior relevant to autism pathophysiology.

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Poster

736. Optogenetic Studies of Neural Circuits

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Hughes Collaborative Innovation Award (HCIA)

Title: Independent circuit wiring and information representation within parallel nigrostriatal dopamine circuits revealed with intact-brain structural and functional analysis

Authors: *T. N. LERNER[1,2], C. SHILYANSKY[3], T. J. DAVIDSON[1,2], K. E. EVANS[4], K. T. BEIER[4,5,6], K. A. ZALOCUSKY[1,2,7], A. K. CROW[2], R. C. MALENKA[4,5], L. LUO[3,6], R. TOMER[2], K. DEISSEROTH[1,2,4,6];
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Abstract: Recent controversy regarding the diversity and connectivity of midbrain dopamine neurons has highlighted the importance, and the challenges, of defining cell types and roles in the mammalian brain. One promising idea is that brain cells are best defined, with their function most fully understood, using inclusive criteria spanning patterns of gene expression, biophysical properties, wiring of inputs, wiring of outputs, and activity during behavior; however linking all five of these measurements to cell types within the intact brain of living mammals has been difficult. Here, using an array of intact-brain circuit interrogation tools including CLARITY, optogenetics, viral tracing, and fiber photometry during behavior, we explore the complexity of dopaminergic cells within the mammalian substantia nigra pars compacta (SNc). We identify two parallel and largely non-overlapping nigrostriatal dopamine neuron subpopulations, separable by distinct biophysical properties (Ih current, DMS-projecting 296.2 ± 29.25 pA, n=21 vs. DLS-projecting 460.8 ± 49.69 pA, n=16, $p<0.01$), input wiring (using a viral tracing approach, TRIO, two-way ANOVA revealed a significant interaction between the starter cell projection target (DMS-projecting vs. DLS-projecting) and the input area to SNc ($p<0.001$, n=4 brains per group)), output wiring to dorsomedial striatum (DMS) versus dorsolateral striatum (DLS) (fraction of DMS-projecting axons located within the DMS 0.98 ± 0.01, n=2, fraction of DLS-projecting axons located within the DLS 0.86 ± 0.02, n=2), and natural activity patterns during free behavior (in this case, discriminated specifically by the nature of response to aversive stimuli; peak ΔF/F during shock, DMS-projecting -2.628 ± 0.513%, n=8 vs. DLS-projecting 1.527 ± 0.358%, n=9, $p<0.0001$). Together, these results reveal independently controlled information representations streaming through the SNc, with implications for understanding the logic of dopaminergic signaling to striatum and the diversity of cell types in the mammalian brain.

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Poster

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Gatsby Foundation

NSF GRFP

Title: Simultaneous multi-fiber photometry calcium recordings from deep brain regions using a single sensor

Authors: *C. K. KIM[1], S. J. YANG[2], N. PICHAMOORTHY[3], I. KAUVAR[2], T. N. LERNER[3], T. J. DAVIDSON[3], C. RAMAKRISHNAN[3], K. DEISSEROTH[4,3,5];
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Abstract: Fiber photometry is becoming a widely adopted technique for measuring the population activity of genetically- and anatomically-defined neurons during free behavior. Fiber photometry's simple instrumentation has the potential to permit simultaneous neural recordings from multiple deep brain regions. However, existing fiber photometry microscopes couple the emission from a single fiber to a single photodetector; this design makes multi-area recordings cumbersome, since every additional fiber requires an additional detector. Here we describe an sCMOS-based fiber photometry microscope capable of monitoring multiple regions simultaneously *in vivo* using only a single excitation source and camera sensor. On this new microscope, intracellular calcium signals (using nuclear-localized GCaMP6) could be readily detected from up to 7 different regions in a freely moving mouse. We considered that the high sensitivity of this system might also allow simultaneous recording of distinct axonal activity signals from multiple independent projection targets of ventral tegmental area dopamine (VTA-DA) neurons; indeed, we were able to record somatic VTA-DA signals while also recording independent axonal projection activity signals in medial prefrontal cortex, nucleus accumbens, and the basolateral amygdala, during administration of a reward or aversive tail shock (n=6 mice with at least two regions exhibiting responses to reward or shock, $p<0.05$ Wilcoxon's Signed-rank Test). This multi-fiber photometry system could also be readily adapted for multi-color observation, or for combined all-optical observation and perturbation of neural activity. Using an image splitter placed in front of the camera, we simultaneously imaged VTA-DA and VTA-non-DA neurons using GCaMP6 and RCaMP-2, and observed similar or opposite activity patterns between the two populations during administration of a reward or tail shock, respectively (n=12 and 10 trials, $p<0.05$ Wilcoxon's Signed-tank Test). Finally, we optogenetically stimulated VTA-DA neural activity with a novel red light-activated channelrhodopsin while measuring

activity using GCaMP6. Using this approach, we could titrate the stimulation light power to elicit GCaMP6 transients of similar magnitude to physiological responses such as those evoked by reward delivery - a key goal of precision optogenetics (optogenetic responses with various light powers ranging from 2.27±0.57 to 22.29±0.28 %dF/F n=2-6 trials, reward response 8.27±1.63 %dF/F n=4 trials). Future modifications to our microscope may allow such combined dual-color imaging and optogenetic perturbation across all 7 fibers.

Disclosures: **C.K. Kim:** None. **S.J. Yang:** None. **N. Pichamoorthy:** None. **I. Kauvar:** None. **T.N. Lerner:** None. **T.J. Davidson:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** None.

Poster

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DARPA Neuro-FAST program

Gatsby Foundation

Title: Next-generation optogenetic chloride channels arising from crystal structure-guided molecular engineering

Authors: ***A. BERNDT**[1,2], S. LEE[2], J. WIETEK[5], C. RAMAKRISHNAN[2], S. IYER[2], S. PAK[3], S. DELP[2], P. HEGEMANN[5], K. DEISSEROTH[2,6,4];
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Abstract: Chloride channel conductances are critical for the natural regulation of neuronal activity, and the recent development of light-gated chloride-conducting channelrhodopsins has enabled optogenetic inhibition of neurons using this physiologically relevant mechanism. Here we build upon our initial crystal structure-guided approach to engineer new enhanced chloride-conducting channelrhodopsins, in the process testing and extending our hypothesis for ion selectivity in channelrhodospin, by changing the surface potential of the ion conducting pore to enhance chloride flux. The best-performing of these new optogenetic tools, iC++, has near-

perfect chloride selectivity resulting in reversal potentials as hyperpolarized as - 78 mV ± 0.7 under physiological conditions, and with >5 times larger photocurrent compared to the first generation of chloride conducting light-activated channels iC1C2 ($P<0.0001$). As a result iC++ shows reliable spike inhibition in cultured neurons which significantly exceeds iC1C2 performance. To test applicability for *in vivo* application, we expressed iC++ in various cell types of the central and the peripheral nervous systems, and observed robust inhibition in fast-spiking hippocampal interneurons, dopaminergic neurons in substantia nigra, cells of the dorsal root ganglion, and cortical pyramidal neurons under controlled current injection-based stimulation conditions tuned to each cell's spike firing threshold. Next, since cytosolic chloride concentrations differ among cell types, subcellular compartments and even developmental stages, to test performance limits we characterized iC++ under widely varying chloride gradients and with extremely strong current injections. As expected, inhibition efficiency was tunable in this way; as with native inhibitory chloride channels, the reversal potential of iC++ was found to precisely follow varying chloride gradients. Under very strong current injections into cortical pyramidal cells (740 pA ± 84, 10 - 20 Hz, 10s), spike inhibition probabilities ranged from 71% ± 13 ([Cl-]int = 4 mM) to 23% ± 17 ([Cl-]int = 20 mM). However, in response to more moderate naturalistic levels of steady depolarizing current (229 pA ± 24, 10 s), iC++ performed at 100% successful spike inhibition even with internal chloride concentrations ranging from 4 mM up to 20 mM. We conclude that iC++ operates as an essentially pure chloride channel much like native inhibitory mechanisms involving endogenous chloride channels such as the GABA-A receptor. The design features of iC++ further illuminate electrostatic and steric structure-function relationships of the channelrhodopsin light-gated pore.

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Poster

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Beckman Institute for Optogenetics and CLARITY

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Title: Labeling membrane proteins *in vivo* and in PACT cleared tissue with genetically-encoded protein tag technologies

Authors: *C. BEDBROOK[1], M. KATO[2,4], S. KUMAR[2], A. LAKSHMANAN[2], N. FLYTZANIS[2], A. J. RICE[3], P. W. STERNBERG[2,4], F. H. ARNOLD[3,5], V. GRADINARU[2]; [1]Bioengineering, [2]Div. of Biol. and Biol. Enginneering, [3]Div. of Chem. and Chem. Engin., Caltech, Pasadena, CA; [4]Howard Hughes Med. Inst., Pasadena, CA; [5]Div. of Biol. and Biol. Engin., Pasadena, CA

Abstract: Many modern neuroscience methods rely on transgenic expression of membrane proteins for controlling or sensing neuronal activity. Visualizing membrane protein localization and trafficking in live cells can facilitate a greater understanding of the molecular basis of cellular dynamics. In addition, tracking projections from neurons with transgenic expression of membrane proteins can aid in understanding the architecture and circuitry of targeted cell populations, especially in the context of large tissue volumes. We present a method for specifically labeling the plasma membrane-localized fraction of heterologous membrane protein expression using channelrhodopsins as a case study. We show that the genetically encoded, covalent binding SpyTag and SpyCatcher pair from the Streptococcus pyogenes fibronectin-binding protein FbaB (Kato et al., 2012) can selectively label membrane-localized proteins in primary cultured neurons and *in vivo* in Caenorhabditis elegans. We use this system to develop a channelrhodopsin membrane localization assay in mammalian cells that is amenable to high-throughput screening for opsin discovery and engineering. We also describe a method for tagging and labeling opsin proteins in fixed, cleared tissue volumes using the SNAP-tag technology (Juillerat et al., 2003) and we validate this labeling technology for tracking neuronal morphologies in PACT / CLARITY cleared tissue. Both the SpyTag/SpyCatcher and SNAP-tag protein-labeling methods offer advantages for live cell and fixed tissue applications when compared with traditional antibody labeling methods. The SpyTag/SpyCatcher method is composed of all genetically encoded parts, which enables expression and labeling of two components within specific cells in live animals. The SpyCatcher labeling protein is inexpensive and easy to produce in bulk using standard protein expression methods. The SNAP-tag method uses small, bright chemical labeling probes, which are able to rapidly penetrate fixed, cleared tissue. Additionally, both the SpyTag/SpyCatcher and SNAP-tag methods allow for easy swapping of spectrally diverse fluorescent molecules enabling flexibility in fluorescence labelling to fit experimental needs.

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Poster

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Beckman Institute for Optogenetics and CLARITY

Pew Charitable Trust

Kimmel Foundation

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Title: Deep brain mapping of functional connectivity in intact circuits via CaMPARI and PACT tissue clearing

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Abstract: Mapping the functional connectivity within neural circuits of model organisms has been a longstanding goal of neuroscience. Efforts to develop tools for real-time imaging of neuronal activity *in vivo* through fluorescent indicators have been fruitful for mapping superficial cortical areas and the recent developments of endoscopes and fiber photometry have also enabled deep brain imaging. However, imaging activity across large brain volumes at depth remains a challenge. Although post hoc measures of neuronal activity such as the staining of cFos and other immediate early genes' (IEGs) products have been used throughout the brain, they are

limited by their poor temporal resolution. Therefore, to provide large-area functional mapping within temporally relevant periods locked to a stimulus or during behavior it is necessary to develop a method that combines the ability of IEG-like labelling to reveal activity changes in circuits across the brain with the temporal resolution of real-time sensors. CaMPARI is a recently developed neuronal activity integrator (Fosque et al., 2015) that uses a photoconvertible calcium indicator to label active circuits in a precise spatial, temporal, and cell type-specific manner. Building on our prior CLARITY work, we have further developed tissue clearing methods to image thick sections of deep brain structures. Here, we combine our passive CLARITY technique (PACT) (Yang et al., 2014) with CaMPARI to demonstrate a method for mapping network activity within deep brain regions in direct response to an excitatory stimulus. We first validate the compatibility of tissue clearing methods with retention of endogenous CaMPARI fluorescence by showing expression throughout thick (> 1 mm) cleared mouse brain sections. CaMPARI uses 405nm photoconversion light, which has poor tissue penetration, potentially limiting the effective volume that could be functionally mapped. Supporting the feasibility of a combined CaMPARI - PACT approach for deep brain circuit mapping, we achieved photoconverted fluorescence up to 1mm away from the fiber optic implant in anesthetized mice. Further development of this technique could be enabling for mapping the temporal progression of neuronal activity in response to stimuli or behavior throughout the brain.

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Poster

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Gatsby Foundation

Title: Retention and detection of RNAs in CLARITY

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Abstract: Integrating information about the identity, function and connectivity of neural networks in intact 3D volumes is a fundamental aim in neuroscience. Recently, several tissue clearing techniques have emerged to enable the visualization and phenotyping of intact neural circuits, but most of these are not readily compatible with RNA labeling, and systematic methodologies to investigate the RNAs present in such volumes have not been described. The ability to reliably detect RNAs is critical for the investigation of cell activity (e.g. using temporally-tuned immediate early gene activity markers), cell identity and molecular signaling (e.g. using coding mRNAs for which antibodies may not exist or be practical, as well as small and large non-coding RNAs), and using RNAs differing in healthy and diseased states (e.g. in psychiatric conditions). Here, we characterize the extent of RNA retention in clarified tissue under varying CLARITY parameters and identify conditions in which RNAs can be preserved in clarified tissue and stably stored for many months. We further characterize the diffusion of different biomolecules into the tissue (DNA oligonucleotides diffuse significantly faster than RNA oligonucleotides, $p=0.04$), determine the rate-limiting steps (antibody-based amplification used in traditional ISH is slow and non-uniform), and implement an all-DNA based detection and amplification strategy for whole-brain RNA visualization. The resulting approach may enable labeling of several targets simultaneously, allowing for broad molecular phenotyping in which the robust structure of the CLARITY hydrogel would permit multiple rounds of hybridization. These advances in RNA detection highlight the versatility of the CLARITY technique and begin to allow for a more complete molecular characterization of intact neural networks.

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Poster

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Support: Pritzker Neuropsychiatric Research Consortium

Title: Quantitative assessment of alternative CLARITY procedures in mouse brain

Authors: *D. M. KROLEWSKI[1], V. KUMAR[1], B. MARTIN[1], R. TOMER[2], K. DEISSEROTH[2], H. AKIL[1], S. J. WATSON, Jr.[1];
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Abstract: The CLARITY technique for visualizing 3-dimensional fluorescence immunohistochemistry (IHC) requires prior polymerization of a brain-infused acrylamide fixative to generate a hydrogel mesh through which lipid is subsequently removed with sodium dodecyl sulfate (SDS) detergent (Chung et al., 2013). The resulting linkage between hydrogel monomers, formaldehyde, and proteins using the original protocol proved challenging for achieving sufficient lipid clearing and antibody penetration in transparent tissue. More recently, these obstacles have been tempered by reducing and increasing acrylamide and SDS concentrations, respectively (Tomer et al., 2014; Yang et al., 2014). However, the degree of neuronal protein preservation related to such condition changes is still not completely understood. To address this, our laboratory has developed previously unpublished alternative acrylamide formulas and performed IHC experiments in passively cleared brain samples. Using both standard confocal microscopy and a CLARITY Optimized Light Sheet Microscopy (COLM) system, we employed multiple software packages and conducted quantitative analyses of neuronal and non-neuronal markers. The results of the present study will serve to further optimize CLARITY brain transparency methods.

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Poster

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Beckman Institute for Optogenetics and CLARITY

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Caltech-GIST

Title: A novel adeno-associated viral vector based approach to deliver sparse multicolor labels to defined cell types for the mapping of intact neural circuits in PACT cleared tissue

Authors: *K. CHAN[1], B. DEVERMAN[1], A. GREENBAUM[1], J. CHO[2], C. XIAO[1], S. KUMAR[1], V. GRADINARU[1];
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Abstract: The vast complexity of neuronal shapes and connections throughout the brain spurred the development of approaches (e.g. Brainbow) that use multicolor labeling to distinguish individual cells within dense genetically defined populations. We have expanded on this concept of multicolor labeling for single-cell analysis by building an adeno-associated viral (AAV) vector-based toolbox of constructs with modular promoters and floxed single monomeric fluorescent protein (XFP) cassettes. By coinjecting a mixture of XFP expressing viruses, we can achieve a wide range of hues that facilitates identification and tracing of single cells and their projections. However, even with multicolor labeling, individual cells can be difficult to discern from a dense population of labeled cells. Therefore, we have coupled our toolbox to a novel AAV capsid variant, AAV-PHP.B, which was engineered for efficient brain-wide neuron and glial transduction through the vasculature. By systemically delivering a low dose (~1e10 vector genomes/mouse) mixture of floxed XFP expressing genomes packaged into AAV-PHP.B, we are able to sparsely label genetically defined cell types for single-cell phenotyping and projection mapping. To facilitate mapping of intact circuits, we use the passive CLARITY technique (PACT) together with high-resolution light-microscopy (via a custom built system that can achieve up to 45 frames per second) to image through multi-millimeter thick tissue samples. We demonstrate the utility of this approach, which combines easily customizable genomes, a novel AAV capsid, tissue clearing, and light-sheet microscopy to trace select neural circuits within the brainstem and basal ganglia.

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Poster

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Pew Charitable Trust

Gordon and Betty Moore Foundation through Grant GBMF2809 to the Caltech Programmable Molecular Technology Initiative

Title: Methods for generating hydrogel-stabilized transparent whole organs and organisms for single-cell phenotyping in both soft and osseous tissues

Authors: *J. B. TREWEEK[1], N. FLYTZANIS[1], K. CHAN[1], A. GREENBAUM[1], B. E. DEVERMAN[1], T.-F. HE[2], A. LIGNELL[2], L. CAI[2], C. C. FOWLKES[3], V. GRADINARU[1]; [1]Div. of Biol. and Biol. Engin., [2]Div. of Chem. and Chem. Engin., Caltech, Pasadena, CA; [3]Computer Sci., Univ. of California, Irvine, Irvine, CA

Abstract: To aid in mapping cellular connectivity and studying tissue microenvironments at the whole-organ level, many tissue clearing protocols have been described over the last decade, including CLARITY, CUBIC, 3DISCO, iDISCO, ScaleA2, and SeeDB. Although these techniques circumvent the need to section, individually process, and digitally reconstruct single-plane images from thin-sliced samples, as in traditional histology, the ability to image and interpret data from intact, transparent samples encounters two major bottlenecks. First, obtaining high-resolution image stacks for thick tissue sections requires lengthy acquisitions. Second, analysis of the corresponding terabytes of raw image data is computationally demanding. Thus, in addition to optimizing clearing protocols for a wide variety of tissues (e.g. bone), our recent work has been aimed at addressing these barriers to the high-throughput analysis of large tissue volumes. Entailing four general steps of fixation, hydrogel embedding, chemomechanical removal of light scattering moieties, and refractive index matching, PACT (PAssive CLARITY Technique), PACT-deCal (PACT deCalcification for bone) and PARS (Perfusion-assisted Agent Release *in situ*), and RIMS (Refractive Index Matching Solution) mounting transform excised organs and whole organisms into optically transparent samples within 1-2 weeks. Aside from granting long-range tract tracing through the rodent brain and body, these clearing methods may also be applied to the super-resolution detection of single-molecule transcripts in smFISH. Since the amine-containing macromolecules and nucleic acid transcripts within a sample are locked into place via their hybridization to a size-adjustable hydrogel scaffold, the tissue-hydrogel

matrix can withstand rounds of delipidation, decalcification, enzymatic digestion, histological labeling, and size fluctuations during clearing and mounting. To more efficiently image the resulting samples at high resolution, we built an affordable light-sheet microscope that allows fast imaging (~ 45 frames/sec), and an immersion chamber that accommodates cleared samples. Finally, we delineated a workflow for handling gigabyte-sized raw data files, wherein we highlight image analysis software packages that we found most capable of performing basic functions for cleared tissue data analysis (e.g., semi-automated tract-tracing, cell-mapping and quantification). We anticipate that, with future advances in automated 3D image analysis and registration for terabyte-sized datasets, tissue clearing will enable whole-body anatomical maps to be rewritten with nanoscale resolution.

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Poster

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Title: Integrated high-resolution, high-throughput structural and functional mapping of large intact nervous systems

Authors: *R. TOMER[1,2], M. LOVETT-BARRON[3,2], L. YE[3,2], A. L. SANBORN[3,2,4], N. YOUNG[3,2], A. CROW[3,2], A.-C. WANG[2], R. BURNS[6], J. T. VOGELSTEIN[7], K. DEISSEROTH[3,2,5];
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Abstract: A major challenge in modern neuroscience is to map brain architecture and activity together, with both datastreams collected at high (wiring-level) resolution while maintaining broad (brain-wide) perspective. Light microscopy-based approaches are emerging as important tools for achieving this goal, largely fueled by developments in tissue clearing, genetic labeling and fast high-resolution microscopy. We recently developed CLARITY and CLARITY Optimized Light-sheet Microscopy (COLM), two methods that together allow rapid high-resolution imaging of entire intact adult mammalian brains. While this integrated approach has already provided unprecedented access to the underlying neuronal architecture of mouse brains, many new challenges have emerged for larger brain samples. Here we report three key advances to address these challenges. First, we report development of second-generation COLM (using multiplexed synchronized image detection and illumination optics), which is up to an order of magnitude faster, while maintaining or improving the imaging resolution, and increased (up to two-fold) imaging depth. This new COLM framework reduces the imaging time of an entire mouse brain from hours to minutes, and make intact tissues twice as large along any or all dimensions accessible for imaging in a practical timeframe; we present a complete characterization of this system with large brain examples from rodent and primate. Second, we report a computational tool and data management framework for analyzing the immense data streams that result. The pipeline includes efficient image registration (using B-splines-based multi-resolution strategies), segmentation (for cell detection and tracing), statistical analysis and visualization for (terabyte-scale) data. Third, we report application of these tools for integrated structural and functional mapping of rodent and fish CNS, in which wiring-level anatomy and functional activity are collected on the same COLM system, enabling highly accurate merging of the two modalities. Together, these tools may provide a robust platform for an integrative brain-wide understanding of nervous system structural and functional architecture.

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Poster

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Pew Charitable Trust

Title: Adeno-associated viruses capable of efficient global transduction of the adult central nervous system identified by *in vivo* cell type-specific capsid selection

Authors: *B. E. DEVERMAN[1], P. L. PRAVDO[2], B. P. SIMPSON[2], A. BANERJEE[2], K. Y. CHAN[2], W.-L. WU[2], S. R. KUMAR[2], B. YANG[2], V. GRADINARU[2];
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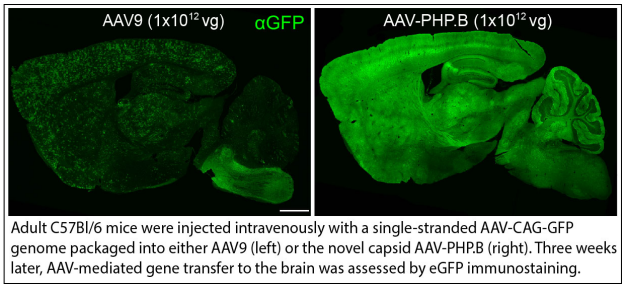
Abstract: Recombinant adeno-associated virus (rAAV) vectors have emerged as essential tools for gene transfer to the central nervous system (CNS). rAAVs are widely used to deliver genetically encoded elements (e.g., opsins, sensors, and RNAi) with high efficiency to discrete CNS regions via intraparenchymal injections. Alternatively, intravascular (IV) administration of certain AAV serotypes, most notably AAV9, can be used for CNS-wide delivery, but the resulting gene transfer efficiency is insufficient for applications requiring transduction of a large fraction of neurons and/or glia, especially in the adult. To achieve efficient gene delivery throughout the CNS, we developed a novel cell type-specific selection platform and used it to identify variants from an AAV capsid library that transverse the vasculature and transduce cells within the CNS. One AAV capsid variant, AAV-PHP.B, when administered IV to adult mice, delivers genes to the brain and spinal cord 40- to 92-fold more efficiently (depending on the CNS region) than the current standard, AAV9. Importantly, AAV-PHP.B efficiently transduces neurons, astrocytes, and oligodendrocytes, making AAV-PHP.B useful as a vehicle for global gene transfer to the brain and spinal cord for a wide variety of applications. We identified AAV-PHP.B, as well as an additional vector AAV-PHP.A, which exhibits enhanced tropism for CNS astrocytes and reduced tropism for non-CNS organs, after only two rounds of *in vivo* selection validating of our novel vector development platform as a powerful approach to customize gene delivery vectors for biomedical applications.



Adult C57Bl/6 mice were injected intravenously with a single-stranded AAV-CAG-GFP genome packaged into either AAV9 (left) or the novel capsid AAV-PHP.B (right). Three weeks later, AAV-mediated gene transfer to the brain was assessed by eGFP immunostaining.

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Poster

736. Optogenetic Studies of Neural Circuits

Location: Hall A

Time: Wednesday, October 21, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 736.15/DD62

Topic: G.04. Physiological Methods

Title: Head-motion modulation of the activity of optogenetically tagged neurons in the vibrissal thalamus

Authors: ***T. B. ORAM**, E. AHISSAR, O. YIZHAR;
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Abstract: The vibrissal system evolved to function in freely-moving animals that explore and interact with their environment. There are two major afferent sensory pathways in the vibrissal system, the lemniscal pathway, which passes through the ventro-posterior-medial nucleus of the thalamus (VPM), and the paralemniscal pathway, which passes through the posterior medial nucleus of the thalamus (POm). To date, it is unclear what the perceptual functions of these pathways are and what type of perceptual information is primarily processed by each. Fortunately, the optogenetic and tracking tools necessary to study the system in a natural-like environment now exist. Conveniently, the lemniscal and paralemniscal pathways are functionally and anatomically segregated at the level of the thalamus. Taking advantage of this segregation, we used a Cre-ON/Cre-OFF genetic targeting strategy to selectively target the expression of channelrhodopsin fused to yellow-fluorescent protein (ChR2-eYFP) to either the VPM or the POm of GPR26-Cre mice, which strongly express Cre in the POm but only sparsely in the VPM. A chronic multi-site optrode was then implanted in the ChR2-eYFP expressing nucleus enabling

(1) recording of the multi-unit and single-unit activity, and the local field potential of awake, freely-moving mice and (2) the assignment of nucleus affiliation to recorded units through "optogenetic tagging". We then recorded the exploratory behavior of freely-moving mice in a walled arena containing a cylindrical object while collecting neural data from nucleus-affiliated single units. Our preliminary data show that the activity of units recorded in both the lemniscal and the paralemniscal pathways (n=10) was modulated by head-motion variables. Modulations depths varied between 0.21 and 0.94 for the different variables (the linear and angular velocity and acceleration of the head, and head orientation), where the strongest modulations were due to the velocity variables (0.94±0.50 for linear velocity, and 0.70±0.36 for angular velocity). Additionally, the firing rate of VPM units was strongly modulated by vibrissal touch events. On average, there were no significant interactions between head- and touch-induced modulations. We are currently collecting data to clarify the sensitivity of identified VPM and POm units to whisking phase and to touch-related variables. Collectively, these data are expected to elucidate the perceptual functions of the lemniscal and paralemniscal pathways in awake, freely-moving mice.

Disclosures: **T.B. Oram:** None. **E. Ahissar:** None. **O. Yizhar:** None.

Poster

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Topic: G.04. Physiological Methods

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Marie Curie CIG grant #321919

Title: Controlling fear associations through optogenetic modulation of amygdala to prefrontal connectivity

Authors: *O. KLAVIR, M. PRIGGE, R. PAZ, O. YIZHAR;
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Abstract: Post-Traumatic stress Disorder (PTSD) is thought to reflect strong and persistent learned fear associations resulting from aberrant synaptic plasticity processes. Despite the extensive study of the neural circuits involved in fear learning, interventions that could reverse pathological associations and ameliorate the debilitating symptoms of PTSD have not been defined. The basolateral amygdala (BLA) and the medial prefrontal cortex (mPFC) play a key role in the acquisition and extinction of fear memories. Here, we establish an optogenetic stimulation protocol for manipulating the synaptic strength of the BLA to mPFC projection, and use this approach to explore this pathway's role in fear learning. Using acute slice recordings and extracellular single-unit recordings in behaving mice, we found that high frequency stimulation (HFS) of BLA projections in the mPFC induced long term depression (LTD) of synaptic strength. This stimulation protocol dramatically reduced the response of mPFC neurons to BLA input without altering the intrinsic properties of mPFC cells or their spontaneous firing rates. We then sought to utilize the unique response to repetitive stimulation of the BLA->mPFC projection to modulate learned fear. We found that depotentiation of BLA->mPFC synapses prior to conditioning leads to impaired fear learning. However, effective treatment should be capable of alleviating the fear response after it had already been acquired. We therefore performed experiments in mice that have already undergone fear learning. In these animals, inducing LTD prior to exposure to the fear-associated cues facilitates extinction learning. Our combined findings suggest a new role for the BLA->mPFC pathway not only in the in the acquisition but also the maintenance of learned fear associations. We also provide an effective way to specifically target and alter the connectivity of the pathway creating a time window for enhanced efficiency of fear extinction.

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Poster

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Marie Curie CIG grant #321919

Title: Strategies for optogenetic silencing of axonal terminals

Authors: *M. MAHN, M. PRIGGE, S. RON, O. YIZHAR;
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Abstract: Light-driven ion pumps are commonly used for temporally precise and effective inhibition of neural activity. The use of such optogenetic tools can potentially allow fast and reversible inhibition of defined projection pathways through illumination of presynaptic terminals. However, the use of optogenetic silencing tools in axonal terminals has not been mechanistically validated. We used two-photon imaging and whole-cell patch clamp recordings in cultured neurons and acute brain slices to evaluate the impact of optogenetic light-driven ion fluxes on the excitability and function of presynaptic terminals. Surprisingly, we found that extended (minutes-long) light-driven activation of a proton pump at presynaptic terminals can lead to accumulation of intracellular calcium and to an increase in synaptic vesicle release. The rate of miniature excitatory postsynaptic currents (mEPSC) increased six-fold within 3 minutes of proton pump activation using moderate light power (2 mW/mm^2), surpassing the average EPSC rate recorded in spontaneously active hippocampal neuron cultures nearly 2-fold. Removal of extracellular calcium during ion pump activation was sufficient to reduce the presynaptic calcium signal, reported by SyGCaMP6s, back to baseline levels. Whole-cell patch clamp recording in acute brain slices from mice transduced with eArch3.0-mCherry revealed an increase in EPSC rate after light application to axonal terminals originating from eArch3.0-expressing cells. These results stress the importance of understanding the unique impact of light-driven ion pumps on the physiology of the presynaptic compartment.

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Poster

736. Optogenetic Studies of Neural Circuits

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NIHR21AT001607

Grace Woodward Foundation Research Grant

Pennsylvania Tobacco Settlement Biomedical Research Grant

Title: Behavioral effects of partial unilateral optogenetic inhibition of the nigrostriatal pathway

Authors: *V. IYER[1], N. PATEL[1], K. VENKITESWARAN[1], E. HANDLY[1], N. IQBAL[1], C. WHITE[1], P. SRIDHAR[1], K. THIAGARAJAN[1], Z. LIU[2], C. RAMAKRISHNAN[3], K. DEISSEROTH[3], T. SUBRAMANIAN[1];
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Abstract: A completely reversible animal model of Parkinson's disease (PD) that is specific to a single neural pathway may benefit our understanding of the pathophysiology of parkinsonism. To test this concept we assessed the effects of unilateral optogenetic inhibition of the nigrostriatal pathway in 18 adult rats. AAV2-Ef1a-mCherry-IRES-WGA-Cre was injected into the left striatum of 14 adult rats while 2 animals served as no virus controls. AAV5-Ef1a-DIO-eNpHR3.0-EYFP was then injected into both left and right substantia nigra (SN) or left SN alone in 11 of the WGA-Cre injected animals while the remaining 3 were WGA-Cre controls. Two animals received only eNpHR3.0-EYFP viral vector into both SN to serve as NpHR controls. Trans-synaptic retrograde transport of WGA-Cre allowed expression of the double floxed eNpHR 3.0 and EYFP and enabled specific targeting of nigrostriatal neurons. A 589 nm laser source and a tethered fiber optic system allowed awake animal behavioral assessments using a rodent behavioral battery of tests (RBBT) comprising the cylinder test, the elevated body swing test, the stepping test and the vibrissae evoked forelimb placement test. The animals were tested with laser intensities ranging from 1.5mW to 5mW. The duration of laser exposure varied from 30 seconds to several hours and was performed both intermittently and continuously. The RBBT was performed at time points ranging from 1 minute after laser exposure to 5 days post laser testing. Experiments were repeated multiple times and compared between pre-laser vs. laser exposure vs. post laser behavioral data on all RBBT tests. Histological examination completed in 9 animals showed excellent targeting and expression of mCherry in medium spiny neurons in the entire left striatum in animals that received WGA-Cre injections. The left SN in test animals showed complete labeling of all tyrosine hydroxylase (TH) positive neurons that project to the left striatum with EYFP and eNpHR. In the same animals the right SN showed a small percentage of interhemispheric neurons expressing EYFP and eNpHR. No EYFP or eNpHR expression was seen in control animals. These techniques allowed differentiation of extrastriatal projections of the SN from its striatal projections. The laser cannula was accurately positioned just above the SN. Remaining histology is ongoing. Our data provides proof of principle that specific Cre mediated eNpHR3.0 and EYFP expression can be achieved in all left SN neurons and all interhemispheric projections from the right SN neurons that make synaptic connections to the left striatum. It also demonstrates the technical feasibility of optogenetic inhibition of the nigrostriatal pathway in rats.

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Poster

736. Optogenetic Studies of Neural Circuits

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Topic: G.04. Physiological Methods

Support: National Agenda Project of the Korea Research Council of Fundamental Science & Technology (NAP-09-04)

KIST Institutional Program(Project No. 2E24721)

Title: Control of bladder function by optogentic modulation on membrane potential of smooth muscle

Authors: ***J. PARK**[1], J. JANG[1,2], J. HONG[1,3], H.-J. MOON[1], H. LEE[1], H. SHIN[1,3], J.-K. SUH[1]; [1]Korea Inst. of Sci. and Techonology, Seoul-City, Korea, Republic of; [2]Dept. of Electronics Engineering, Col. of Engin., Ewha Womans Univ., Seoul, Korea, Republic of; [3]Dept. of Biomed. Engin., Univ. of Sci. and Technol., Daejoen, Korea, Republic of

Abstract: The function of lower urinary tract which is regulated by a complex neural circuit with smooth and striated muscle is subject to be disrupted by various disease and injuries. Conventional treatments for lower urinary tract disorder (LUTD) such as pharmacological agents and electrical stimulation have a limitation in application, due to lack of spatial and temporal accuracy of the treatments. In this study, we show an approach that addresses the two issues of the treatments for LUTD by using optogenetics to modulate membrane potential of smooth muscle cells. Channelrhodopsin (ChR2) and halorhodopsin (Halo) were expressed in smooth muscle cells of mouse bladder through cre-loxp transgenic system. In order to test the feasibility of optogenetic modulation on bladder function, we set up a laser stimulation and pressure recording system for the whole bladder. Whole bladder connected to a pressure transducer via a polyethylene tube was submerged in carbonated (5% CO_2 and 95% O_2) physiological saline solution (pH 7.3~7.4) maintained at 37℃. Each of blue (473nm, ~1mW/mm^2) and yellow laser (589nm, ~3mW/mm^2) was uniformly illuminated to the bladder lumen using a spherical lens. We observed that ChR2 activation by blue illumination evoked bladder contraction and Halo activation by yellow illumination suppressed both electrically induced bladder contraction and

overactive bladder symptom induced by PGE_2 treatment (50μM). We first demonstrated that optogenetic control of membrane potential of smooth muscle cells can bi-directionally regulate bladder function. Considering a fine spatio-temporal resolution of our approach, the optogenetic modulation on bladder may be an effective strategy to restore bladder dysfunction.

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Poster

736. Optogenetic Studies of Neural Circuits

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Topic: G.04. Physiological Methods

Support: NIHR01NS42402

NIHR21AT001607

Grace Woodward Foundation Research Grant

Pennsylvania Tobacco Settlement Biomedical Research Grant

Title: Immunological response to optogenetic laser stimulation of fetal ventral mesencephalic transplants

Authors: R. E. THOMAS[1], *K. VENKITESWARAN[1], E. HANDLEY[1], M. DAWSON[1], C. WHITE[1], A. HARRIS[1], A. STULL[1], C. RAMAKRISHNAN[2], K. DEISSEROTH[2], T. SUBRAMANIAN[1];
[1]Neurol & Neural & Behav Sci., Penn St Hershey Med. Ctr. & Col. Med., Hershey, PA; [2]Dept. of Bioengineering, Stanford Univ., Stanford, CA

Abstract: Optogenetics can potentially be used for precise neuromodulation of central nervous system (CNS) grafts and to control any deleterious consequences of CNS transplants. One potential optogenetic method to precisely control CNS graft function is by the use of a dual vector system that requires graft host synaptic connectivity. We tested the effects of the dual vector system AAV2- Ef1a-mCherry-IRES-WGA-Cre and AAV5-Ef1a-DIO-eNpHR3.0-EYFP in CNS grafts. The WGA-Cre vector was injected to striatum of adult hemiparkinsonian rats and eNpHR3.0-EYFP vector was introduced into E13.5 mouse fetal ventral mesencephalic (mFVM) tissue prior to stereotactic transplantation into the left striatum. Control grafts did not receive any

viral vectors. All animals were treated with cyclosporine post transplantation for a 3-month period. Behavioral effects of these transplantation experiments have been previously reported (Subramanian, T., et. al., SfN Program 295.08/A29. 2014). Detailed histology was performed on all animals at the end of behavioral experiments. Excellent mCherry expression was seen in the entire left striatum indicating exceptional transduction of WGA-Cre vector. Excellent survival of mFVM grafts was also confirmed with TH immunohistochemistry and the cell survival of grafts was quantified using design based unbiased stereology. Graft host connectivity was confirmed by the presence of EYFP and eNpHR positive grafted neurons and in their newly formed axons making synaptic contact with mCherry positive medium spiny neurons in the host striatum. The immune response to these transplants were assessed immunohistochemically using antibodies against glial fibrillary acid protein (GFAP), Ox-42, a marker for activated rat microglia and Ox-6, a marker for MHC class II expression. Our results showed mild immune response as evidenced by GFAP positive activated astroglia, and Ox-42 and Ox-6 positive activated microglia in the vicinity of the grafts. The contralateral hemisphere, showed no expression of these immune markers indicating a mild localized immune response that did not negatively impact graft survival or maturation. Densitometry results showed no significant differences in immune response between control animals that did not receive any optogenetic viral vectors and test animals that received dual optogenetic viral vector exposure. Laser use in these animals also had no apparent negative immune response. These data provide support to the notion that optogenetic viral vectors can be safely used in CNS transplantation experiments and these methods can potentially have application in translational research for PD.

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